JOURNAL OF AGRICULTURAL RESEARCH

VOL. 39 WASHINGTON, D. C., AUGUST 1, 1929 No. 3

A STUDY OF DECAY IN CITRUS FRUITS PRODUCED BY INOCULATIONS WITH KNOWN MIXTURES OF FUNGI AT DIFFERENT CONSTANT TEMPERATURES[1]

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INTRODUCTION

In making a survey of the forms of decay occurring naturally in the field and packing house one is impressed by the fact that the symptomatology of the various forms of rotting under such conditions presents a somewhat different picture from that of the decays induced artifically by inoculations with single pure cultures in the laboratory.

Since the inoculation of fruit with known mixtures of different species of fungi appeared to be a somewhat unexplored field (the majority of investigators having followed the classic path of single pure-culture inoculations), and since it was a method more nearly approximating the natural conditions for infections in the field, a series of differently combined inoculations as well as inoculations with the same organisms singly has been tried for the purpose of obtaining a better understanding of what actually happens in the field and in the packing house. The effect of different constantly maintained temperatures on the development of decay resulting from these inoculations with fungi singly and in various combinations of species was also investigated. Some previous investigation of the temperature relations of decay produced by *Penicillium italicum* and *P. digitatum* inoculated separately were made by Fawcett and Barger (*4*).[4]

It has been a common observation that two or more organisms may be associated in the same lesions and that in many cases the diseases appear to be made more severe by such association. Previous experiments with citrus fungi by Fawcett (*2, 5*) showed that certain mixed inoculations into citrus bark caused much more severe injury than any inoculation with a single fungus of the same group. For example, the injury caused by a mixed inoculation of *Diplodia natalensis* and *Colletotrichum gloeosporioides* was much greater than that caused by inoculations with either fungus alone. Inoculations with *Phytophthora citrophthora* combined with *Fusarium* sp. in California showed that the lesions of *Pythiacystis gummosis* progressed faster than when *P. citrophthora* was inoculated alone (*3*). On the other hand, one organism may hinder the attack or invasion of the other. For example, mixed inoculation by Fawcett (unpublished

[1] Received for publication Jan. 15, 1929; issued August, 1929. Paper No. 204, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station.

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[3] Assistance in carrying on the technic of the experiments was given by Louis L. Huillier.

[4] Reference is made by number (italic) to "Literature cited," p. 196.

data) of *Dothiorella gregaria* and *Pseudomonas juglandis* failed to cause appreciable injury, while a pure culture of *D. gregaria* produced large lesions when inoculated under the same conditions.

MATERIAL AND METHODS

The following species of fungi were isolated just previous to their use in the investigation, from fresh material, by the senior author; the numbers added are the stock culture numbers of the junior author at the Citrus Experiment Station, Riverside, Calif.: *Penicillium italicum* (1437) from lemon; *P. digitatum* (1438) from lemon; *Botrytis cinerea* (1439) from lemon; *Aspergillus niger* (1440) from orange; and *Sclerotinia libertiana* (1441) from lemon. The remainder listed below were cultures which had been isolated some months previous to their use, but were inoculated into fruits and reisolated in order to renew their vigor for the tests: *Oospora citri-aurantii* (1342) from lemon; *Pythiacystis citrophthora* (1309–A) from lemon bark; *Trichoderma lignorum* (1227–L) from lemon; *Alternaria citri* (1372–F) from orange; *Phomopsis californica* (1227–O) from lemon; *Diplodia natalensis* (1241–B) from grapefruit and *Dothiorella ribis* (1232–G) from lemon. All of these had been isolated from California fruits except *D. natalensis*, which came from a grapefruit grown in the Isle of Pines, Cuba. Glucose-potato agar was used for growing all cultures employed in the inoculation tests.

Several different preliminary experiments were made for the purpose of establishing the most satisfactory method of obtaining on the fruit a wound of uniform diameter and depth. At first a small-sized cork borer 4 millimeters in diameter, with a glass tube so placed over it as to limit the penetration to a certain determined point in the fruit, was used. Later, in order to facilitate sterilization, an all-metal instrument of the same size and diameter was made by attaching a metal shoulder around the outside of the cork borer instead of the glass tube. A metal wire was placed inside of the cork borer for the purpose of eliminating the disk of rind after each wound was made. With this instrument one could make uniform wounds in the surface of as many as 600 fruits in an hour. During the experiment the cork borer was sterilized by dipping it in 95 per cent alcohol and flaming after use on each group of 5 to 10 fruits. Before wounding, the fruit was washed and then disinfected with 95 per cent alcohol, which was rubbed over the surface by means of cotton and allowed to evaporate. The use of mercuric chloride was avoided because small amounts left on the rind might check the growth of the inoculum. The disinfection by alcohol proved quite satisfactory and allowed very little contamination, as shown by the almost complete absence of decay in the checks.

For the inoculation of the fruit, glass rods of uniform size with round, sanded ends were used. Frequently during inoculations the rod was dipped in alcohol and cooled before it was dipped into the suspension of spores.

Since it was difficult to avoid the possibility of some variation in the number of spores per inoculum, a preliminary investigation was made to find out what influence certain variations might have on the rate of decay. Six different series of inoculations with *Penicillium italicum* were tried, 20 oranges being used in each series. A spore

suspension containing approximately 2,000,000 spores per cubic centimeter of *P. italicum* was obtained by adding 10 cubic centimeters of sterile water to a 48-hour culture on a glucose-potato agar slant. The dilutions used were 1:1, 1:4, 1:5, 1:8, and 1:16 with sterile water. The average measurements taken every 48 hours (fig. 1)

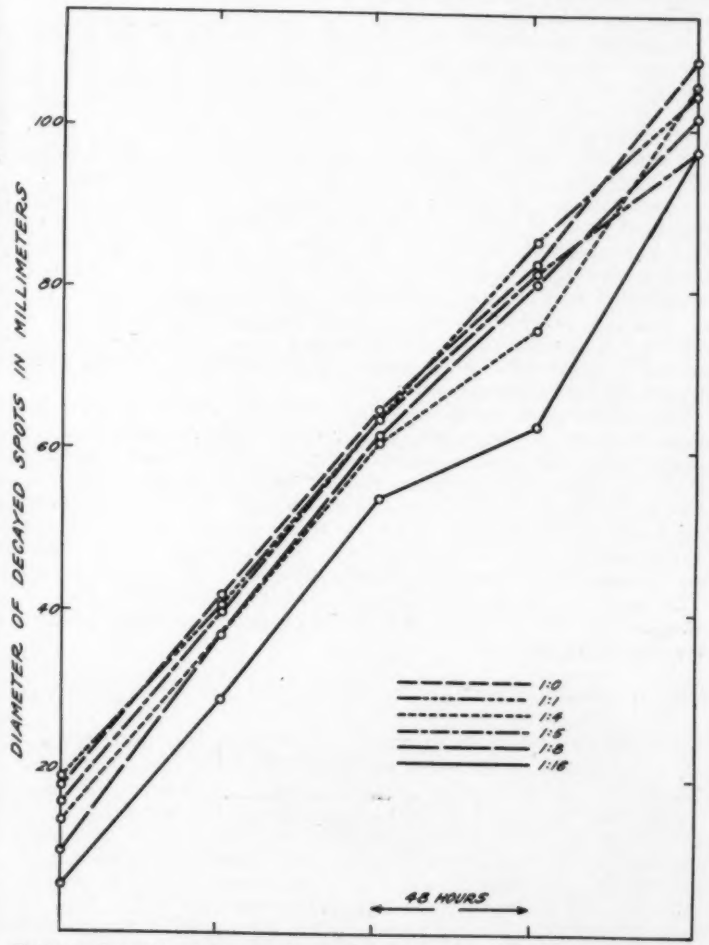

FIGURE 1.—Rates of decay of oranges after inoculations with different dilutions of a spore suspension of *Penicillium italicum*. The standard suspension contained about 2,000,000 spores per cubic centimeter

show that only when the inoculum was diluted sixteen times the volume of the original suspension was there an appreciable decrease in the rate of decay. In all other cases, as Figure 1 indicates, the slight differences in initiation of decay are related to the extent of dilution, but after four days there appears to be no definite rela-

tion between rate of decay and dilution. These suspensions represented a variation from about 2,000,000 to 125,000 spores per cubic centimeter. The experiment seems to indicate that within certain limits the number of spores in the inoculum has no deciding influence on the rate of decay. The density of spores was sufficient to start decay in all the fruits, so that the question of initiation of decay need not be considered.

In all the subsequent inoculations the density of the suspension of spores was so great that any ordinary variation in the inocula was probably not sufficient to make any difference in the rate at which decay proceeded. In mixed inoculations equal amounts of the original spore suspension of each fungus were shaken together. It was therefore concluded that the methods of inoculation used were probably sufficiently uniform to obviate large variations from this source.

The temperature apparatus used during the experiment was an improved type of that described by Livingston and Fawcett (*7*). The improvements consisted in larger temperature chambers with more nearly perfect insulation and the substitution of an electric refrigerator as a cooling unit in place of an ice compartment at one end, and of driving rods with beveled gears in place of wheels and belts underneath for the stirring apparatus. The entire apparatus was located in a basement room where temperature fluctuations throughout any 24 hours were small.

The temperature conditions were surveyed every time the inoculated fruit was to be taken out for observation, generally every 48 hours. Only the average temperature for the duration of each experiment is given. The variation during most of the experiments was from a few tenths to a half degree centigrade. The maximum variation during a few experiments when the apparatus was not in perfect running condition was about 1.5° C. The humidity of the chambers was also recorded in each experiment after the fruit was taken out.

To ascertain to what extent the humidity fluctuated during the complete process of rotting, a series of observations were taken every two days. (Table 1.)

TABLE 1.—*Relative humidity of different temperature chambers used in experiments on citrus-fruit decay*

Temperature of chamber	Relative humidity of chamber—	
	Four days after fruit was inoculated	Six days after fruit was inoculated
° C.	*Per cent*	*Per cent*
6.5	60	64
13.0	74	75
18.0	75	75
22.5	76	78
27.5	71	81
30.5	75	80
32.5	64	55

The variation in humidity in any of the chambers was probably not so great as to be an important factor in influencing the rate of decay. Since the fruit was wrapped and placed in paper sacks, the

humidity conditions surrounding it probably approached those surrounding packed fruit in cars on the way to market. In no case was the humidity so low as to allow noticeable wilting or excessive drying-out of the fruit during the period of the experiment.

EFFECT OF WATER LOSS ON RATE OF DECAY

In seasons when brown rot is prevalent or the fruit is unusually dirty and hot water is used for washing, it has been considered a good packing-house practice to hold fruit two or three days after picking before putting it into the hot water. This is known to prevent it from being injured by the hot water. The fruit thus held loses part of its turgidity, mostly because of water loss. The question arose as to what influence this might have upon the rate of decay. Apart from the question of external resistance to penetration of the fungus, would this loss of water give to the fruit some internal resistance to the progress of the fungus? An experiment designed to

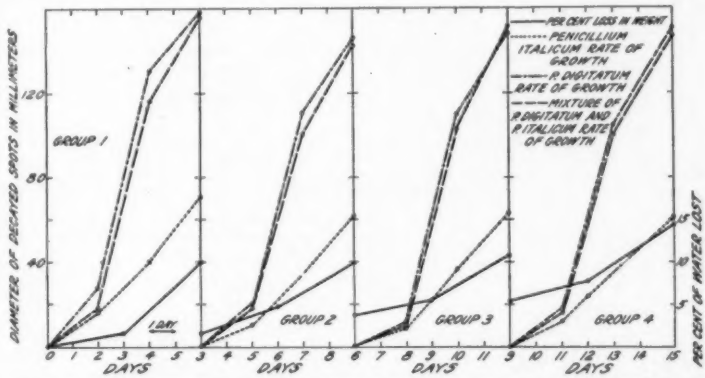

FIGURE 2.—Rate of decay and water loss of lemons inoculated with *Penicillium italicum* and *P. digitatum*, in relation to the period elapsing between the time of picking and of inoculation: Group 1 inoculated soon after picking; Group 2 on the third day; Group 3 on the sixth day; and Group 4 on the ninth day after picking

throw light on this point was performed as follows: A quantity of lemon fruits soon after picking was divided into four groups. The fruits of the first group were inoculated at once, those of the second in 3 days, those of the third in 6 days, and those of the fourth in 9 days. In each group there were 3 lots, 1 of 15 fruits inoculated with *Penicillium italicum*, 1 of 15 fruits inoculated with *P. digitatum*, and 1 of 20 fruits inoculated with a mixed spore suspension of the two fungi. These fruits were kept in a basement room at about 72° F. (22.2° C.) and about 43 per cent relative humidity during the experiment. After inoculation, the fruits of each group were placed in paper sacks. A careful record was taken every three days of the loss in weight of each group from picking until complete rotting of the fruit. This loss of weight is considered as loss of water. A record of the progress of rotting for each group was taken every 48 hours. The graphs shown in Figure 2 indicate that, with the exception of the group inoculated soon after picking, the rate of decay was almost the same whether the fruits were inoculated on

the third, sixth, or ninth day. The group inoculated soon after picking showed a rate of decay somewhat higher than the others. The total loss of water from picking to inoculation and during the period of rotting of the different lots is given in Table 2.

TABLE 2.—*Loss in weight (water loss) of groups of 50 lemons each, the rate of decay of which is shown in Figure 2*

[The fruits of Group 1 were inoculated immediately after picking; those of Groups 2, 3, and 4 were inoculated on the third, sixth, and ninth days, respectively, after picking]

Number of days after picking	Percentage loss in weight of lemons in—			
	Group 1	Group 2	Group 3	Group 4
0				
3	1.5	1.5	1.8	1.7
6	10.0	4.7	3.6	3.6
9		10.0	6.5	5.4
12			10.8	7.8
15				14.3

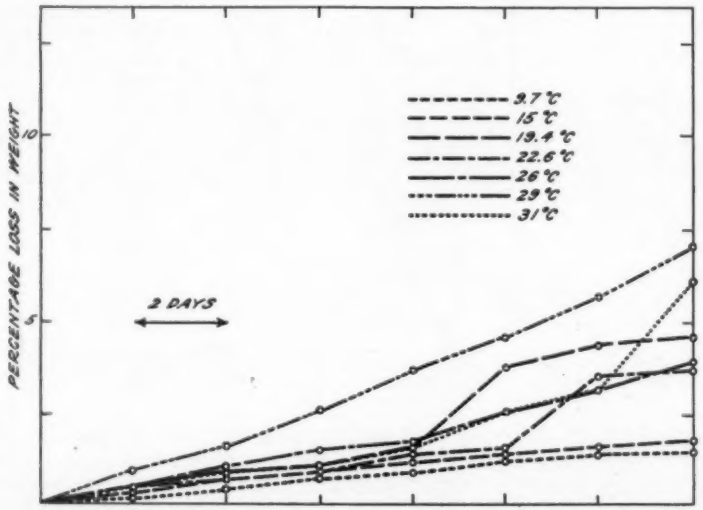

FIGURE 3.—Percentage of loss in weight on bases of initial weight in seven lots of yellow lemons held 14 days at 87 per cent humidity, and at varying temperatures, preceding the inoculations. (See also fig. 4)

EFFECT OF PREVIOUS CONSTANT TEMPERATURES ON RATE OF DECAY

To obtain some idea of the effect of the previous temperature conditions on subsequent rate of decay the following experiment was performed. Yellow lemon fruits directly from the field were divided into seven groups of 50 lemons each, uniform in size and color. These groups were weighed separately and put into different chambers of the temperature machine. Every two days a careful record was taken of the loss in weight of each group, constantly kept at the same temperature.

From the graphs in Figure 3 it is seen that the loss in weight of this fruit before inoculation increased with the temperature except at the highest one, 31° C.

After the fourteenth day the fruit was taken from the different chambers and left for 24 hours in the basement until it reached the temperature of the room, 22.4° C. (72.3° F.). The fruits were then inoculated with *Penicillium italicum*, with *P. digitatum*, and with a mixture of the two, and all were left in the basement at an air temperature averaging 22.4 and a humidity outside the bags of 42 per cent. The increment of rotting was measured every day for nine days, starting from the third day.

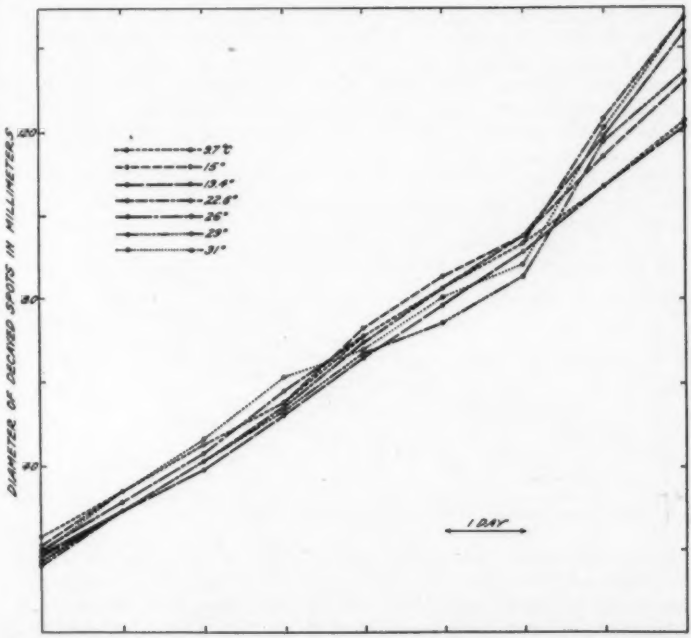

FIGURE 4.—Rate of decay of seven lots of yellow lemons (tree-ripe fruit) inoculated with *Penicillium italicum* and held at 22.4° C. Just prior to inoculation the lots had been subjected to 87 per cent humidity, at the temperatures indicated, for a period of 14 days

The graph for *Penicillium italicum* (fig. 4), which is representative of the three lots, seems to show that there is no definite relation between the previous exposure to different temperatures for an initial period of two weeks from picking and the subsequent rate of decay from uniform inoculation.

EFFECT OF ALTERNATING TEMPERATURES ON RATE OF DECAY

The question arose during the investigation as to the possible effect on decay of alternating temperatures as compared to constant temperatures.

Experiments by McKinney (*8*) had shown that for *Helminthosporium sativum* the effect of an alternating temperature, low during

the night and higher during the day, was practically equal to a constantly maintained temperature that was near the average of the two extremes. To make a comparison of this kind between constant and alternating temperatures, the following experiment was performed. The fruit from the field was left in the basement for 24 hours to acquire the room temperature, about 21° C. The next day two groups of lemons, each of three lots, were inoculated with *Penicillium italicum* alone, *P. digitatum* alone, and a combination of the two. The fruit was wrapped in regular citrus paper, each group put in a separate paper bag and then placed in chambers, one at 9.8° and the other at 27.1°. One group was left undisturbed in the two chambers as a check; the other was alternated every 12 hours from the highest to the lowest temperature chamber, and vice versa. Another check was left in a chamber at 20°, which temperature was nearest to the average of the two extremes. Measurements of the increment of rotting of the fruit were taken every 12 hours, except the first measurement, which was taken after 24 hours. The lots of fruit inoculated respectively with *P. italicum* and *P. digitatum* which

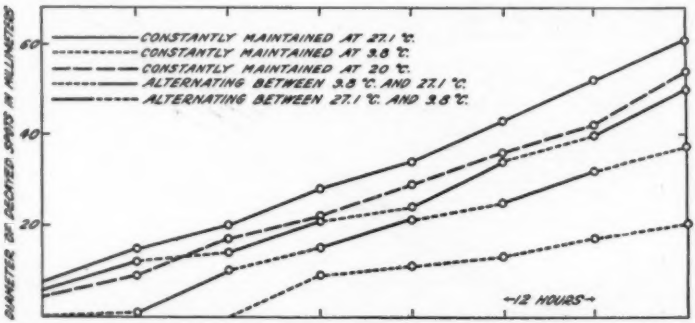
CONSTANTLY MAINTAINED AT 27.1 °C.
CONSTANTLY MAINTAINED AT 9.8 °C.
CONSTANTLY MAINTAINED AT 20 °C.
ALTERNATING BETWEEN 9.8 °C. AND 27.1 °C.
ALTERNATING BETWEEN 27.1 °C. AND 9.8 °C.
DIAMETER OF DECAYED SPOT IN MILLIMETERS
60
40
20
12 HOURS

FIGURE 5.—Rate of decay of lemons inoculated with *Penicillium italicum* and stored at alternating temperatures as compared with rate of decay when stored at constant temperatures

had been alternated between 9.8° and 27.1° C. after an initial exposure of 24 hours to the higher temperature of 27.1°, showed average rates of decay not very far from those of the fruits that had remained in a chamber at 20° and were not alternated. On the other hand, the lots which were alternated between the same 9.8° to 27.1° after an initial exposure to the lower temperature, 9.8°, showed rates of decay below those held at a constant temperature of 20°. This is illustrated in Figure 5 for *P. italicum*.

The slope of the graphs for rate of decay of the fruits in alternated temperatures change with each change of temperature and become almost parallel with the slopes of the corresponding graphs at the same temperature as the fruits not alternated.

LAG IN FRUIT TEMPERATURES AFTER INOCULATION

The time required for the temperature of oranges and lemons to come to the temperature of the chambers was tested to determine the effect of the lag in temperature on initial decay. The first test was made with average-sized lemons about 150 millimeters in circum-

ference, unwrapped. The fruit, carefully selected for size and color, was divided into two lots. In one lot thermometers were inserted in the center of the fruit and in the other they were inserted under the rind. The thermometers were sealed in the fruit with paraffin to prevent air from entering along the stem. The fruit was left in the extreme chambers, 49° F. (8.3° C.) and 88° F. (31.1° C.) overnight until the two lots were found to have attained the temperature of the respective chambers. The two lots were then interchanged—the lot from the higher temperature chamber was removed to the lower temperature chamber, and that from the lower temperature chamber to the higher.

The temperature just under the rind changed somewhat more rapidly at first than that in the center of the fruit, but after three to four hours both were the same, and in five hours the two lots had attained the temperature of the chamber in which they were placed.

In another experiment the fruit was wrapped and placed in paper sacks, as was done in most of the subsequent inoculation experiments. In one group the thermometers were inserted through the wrapper just inside the rind and in the other group they were inserted in the center of the fruits. The initial temperature of all the fruit was about 26.5° C. Different lots were placed in chambers at temperatures of 9°, 20.5°, 28°, and 34.5° C., and the temperature at intervals of two hours was recorded. Table 3 shows the results of this test. Differences of temperature of from 6.5° to 8° C. between the interior of the wrapped and bagged fruit was overcome completely in 6 to 10 hours, and a difference of 17.5° was overcome in 12 hours.

TABLE 3.—*Change in temperature of wrapped lemons exposed in chambers at four different air temperatures*

[Initial fruit temperature, 26.5° C.]

Temperature of chamber (° C.)	Position of thermometer in fruit	Temperature of fruit after exposure for—					
		2 hours	4 hours	6 hours	8 hours	10 hours	12 hours
		° C.	° C.	° C.	° C.	° C.	° C.
9.0	At center	18.0	14.5	12.0	11.0	10.0	9.0
	Just under rind	17.0	14.0	12.0	11.0	10.0	9.0
20.5	At center	23.5	22.5	20.5	20.5	20.5	20.5
	Just under rind	22.5	21.5	20.5	20.5	20.5	20.5
28.0	At center	26.5	27.0	27.5	27.5	28.0	28.0
	Just under rind	26.5	27.0	27.5	27.5	28.0	28.0
34.5	At center	30.0	32.0	33.0	34.0	34.5	34.5
	Just under rind	32.0	33.0	34.0	34.5	34.5	34.5

COLOR, RESISTANCE TO PUNCTURE, AND RATE OF DECAY

Observation had indicated that green lemon fruits decay less rapidly than yellow ones. From about 600 lemons of the same size, eight groups were selected showing gradations of color from deep green to deep yellow. (Table 4.)

TABLE 4.—*Approximate color* [a] *of eight groups of lemons before and after inoculation with Penicillium italicum*

Group No.	Color before inoculation	Color after the last observation
1	Green yellow	Mustard yellow.
2	Greenish yellow	Strontian yellow.
3	Lemon yellow	Do.
4	Lemon chrome	Aniline yellow.
5	Cress green	Amber yellow.
6	Light cress yellow	Do.
7	Absinthe green	Primuline yellow.
8	Citron green	Lemon chrome.

[a] According to Ridgway's Color Standards (9).

After the resistance to puncture was measured with a penetrometer (6) each fruit was inoculated with *Penicillium italicum*.[5] The fruit was then left at a temperature of 70° to 75° F., and the rate of growth of the fungus was recorded every 48 hours. The graphs representing the averages of each group (fig. 6) show a close relation between rate of decay and resistance to penetration and also between rate of decay and color.

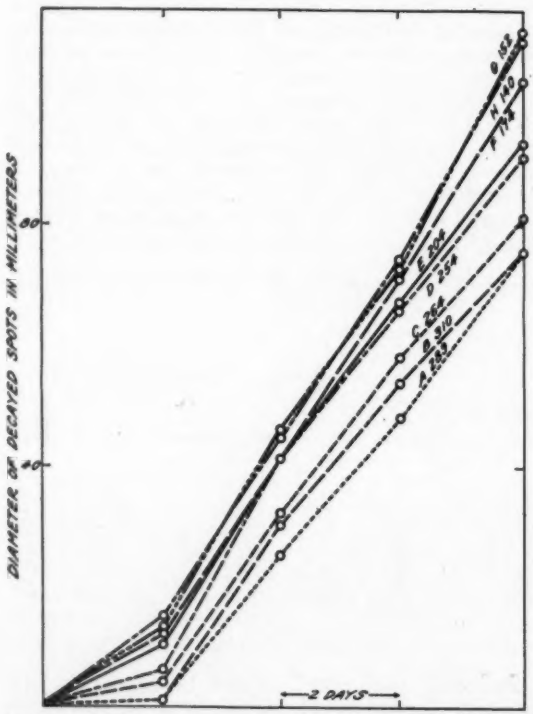

FIGURE 6.—Rate of decay of lemons as related to their color from dark green (A) to light yellow (H), and to resistance to penetration. The resistance to pressure as measured with a penetrometer and expressed in grams averaged as follows for lemons in each group: A, 289; B, 310; C, 264; D, 254; E, 204; F, 174; G, 152; H, 140

The relationship between resistance to penetration and rate of decay is shown by the graphs in Figure 7 when the individual fruits were divided into groups differing by 50 grams of pressure without reference to color. Here the average rate of decay of the different groups is distinctly decreased as the resistance to pressure increases. The experiment indicated that color was a fairly good criterion for the selection of fruit with approximate uniformity of resistance to pressure and also uniformity of

[5] These measurements were made with the help of E. R. Parker, of the Citrus Experiment Station.

resistance to rate of decay by *P. italicum*. Fruits for the different sets of subsequent experiments were selected for approximate uniformity of size and color in order to avoid undue individual variability.

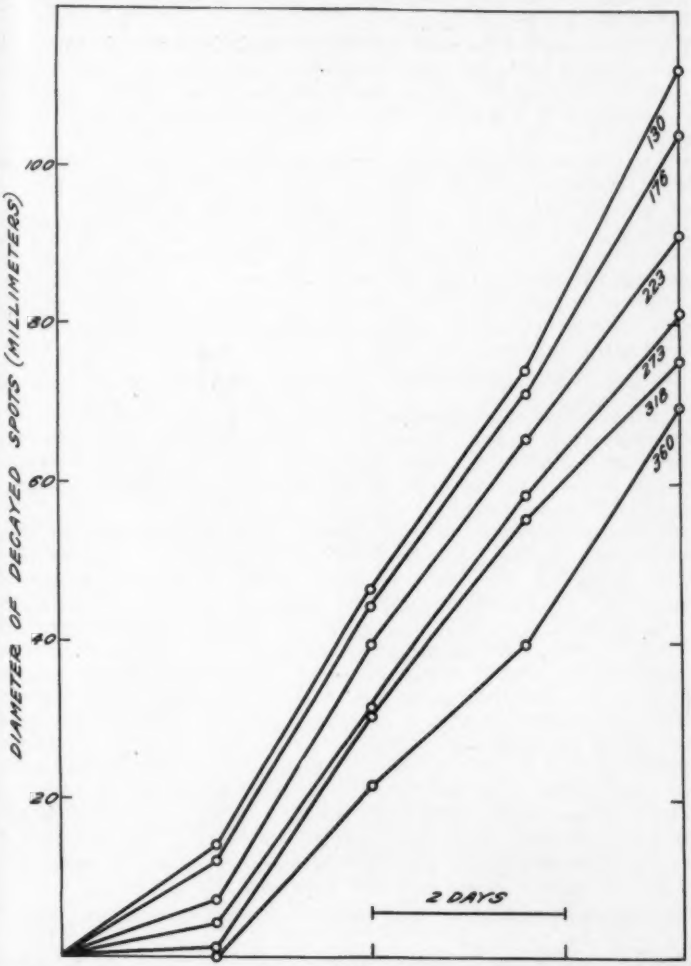

FIGURE 7.—Rate of decay of lemons as related to pressure (expressed in grams) necessary to puncture the rind

COMBINED INOCULATIONS OF PENICILLIUM ITALICUM AND P. DIGITATUM

Oranges and lemons were inoculated as previously described with a mixed suspension of spores of *Penicillium italicum* and *P. digitatum*. For comparison a second group of fruits was inoculated with *P. italicum* alone, a third with *P. digitatum* alone, and a fourth was injured only as a check. The first group contained 70 lemons and 70

oranges divided into seven equal lots of 10 lemons and 10 oranges, and a lot was placed at each of the seven temperatures shown in Table 5. The second, third, and fourth groups contained 35 lemons and 35 oranges divided into lots of 5 lemons and 5 oranges and distributed like the first group.

The measurements in millimeters were made from margin to margin of the decayed spots through the place of inoculation. The fraction of a millimeter resulting from the computation of the averages was not considered if it was less than one-half millimeter, if more it was considered as unity.

During the experiment several points were taken into consideration, namely, sporulation of the fungi inside and outside the fruit, appearance of mycelium on the surface, discolorations during the process of rotting inside and outside of the fruit, general differences between the series of combined inoculated fruit and the other series inoculated with single organisms; and isolation of the fungi in the combined inoculation series from day to day to determine whether or not the rotting tissues inside the fruit contained the same fungus as that shown on the outside.

APPEARANCE OF MYCELIUM ON THE SURFACE OF THE FRUIT AND SPORULATION

In both lemons and oranges *Penicillium italicum* sporulated at a lower temperature than *P. digitatum*. The sporulation range of the former was from 9.8° to 29.8° C. (most abundant spores at about 20°) and of the latter from 17.8° to 29.8° (most abundant spores at about 27.6°) in eight days.

In the combined inoculations of lemon, the mycelium of both fungi was observed on the same fruit. The blue spores of *Penicillium italicum* appeared at a lower temperature than the green spores of *P. digitatum*, which occurred at no temperature below 17.8° C. in the eight days of the experiment. Above this temperature the sporulation of *P. italicum* was confined to a few millimeters around the inoculation wound, while sporulation of *P. digitatum* became abundant in a few days over the remainder of the decayed areas. In the cases studied the area of mycelium of *P. digitatum* as compared to the size of the spot diminished as the distance from the optimum temperature (about 25°) for rate of decay increased. In the combined inoculation, sporulation of *P. italicum* was slightly inhibited by the presence of *P. digitatum*. As decay due to *P. italicum* advanced, sporulation inside at the core was noted. No sporulation of *P. digitatum* inside the fruit was observed. At all temperatures used the rot started in the rind and advanced into the pulp.

DISCOLORATIONS DURING ROTTING AND DIFFERENCES IN COMBINED AND SINGLE INOCULATIONS

Both in oranges and in lemons in all the series inoculated, whether with a mixed or a single inoculum, there appeared at temperatures above 21° C. a brown marginal discoloration 5 to 15 millimeters in width at the extreme advancing margin of decay. At the highest temperature the color was darker than at other temperatures; the lemons were clove brown and the oranges were sayal brown (Ridgway (*9*)). The discoloration seemed to have been produced by the higher temperatures.

The combination of the two species of Penicillium produces a characteristic reddish or pinkish discoloration on and in the rind

around the inoculation point for 5 to 10 millimeters. This kind of discoloration has never been found in inoculations of *Penicillium italicum* or *P. digitatum* alone. When such fruits are cut through the inoculated wound there is found a reddish discoloration of the tissue underlying the rind. (Fig. 8.) This discoloration is an aid in identifiying mixed infections of these fungi, as has been pointed out by Fawcett and Lee (*5*).

As is shown in Table 5, in which are compared the rates of growth on the fourth and sixth days, the combined inoculation in lemons produced an increased rate of decay (fig. 9) at the low temperatures

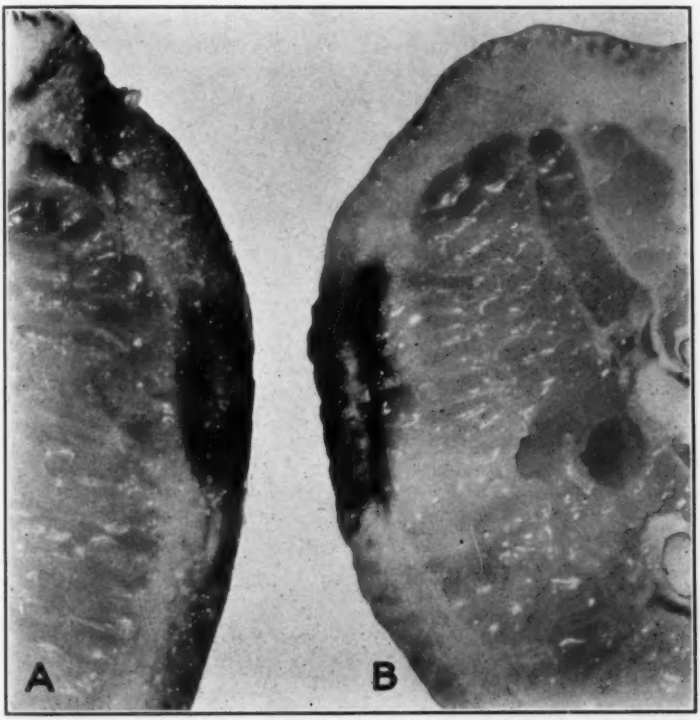

FIGURE 8.—Decay in inoculated fruit; the dark portion indicates the red discoloration typical of decay after a mixed inoculation with *Penicillium italicum* and *P. digitatum:* A, In lemon; B, in orange

9.8° and 14.1° C. and at the high temperatures 27.6 and 29.8°, as compared with *Penicillium digitatum*, but slight differences at intermediate temperatures. In oranges there is an indication that the rate of growth of the mixture is the same or slightly lower than that of *P. digitatum* alone. To determine whether or not the same fungus was present in the interior of the fruit as appeared from the sporulation on the surface, a series of daily isolations were made as shown in Table 6. In cases where more than one organism occurred in the cultures the first mentioned was the most prominent. In making such cultural tests the following method was used. Ten lemons and

10 oranges were inoculated on the same day with *Penicillium italicum* and *P. digitatum* and kept at 21.5° C. (71° F.). Every 24 hours two oranges and two lemons were taken for isolations. Before these were cut they were washed for 10 minutes in tap water to carry away

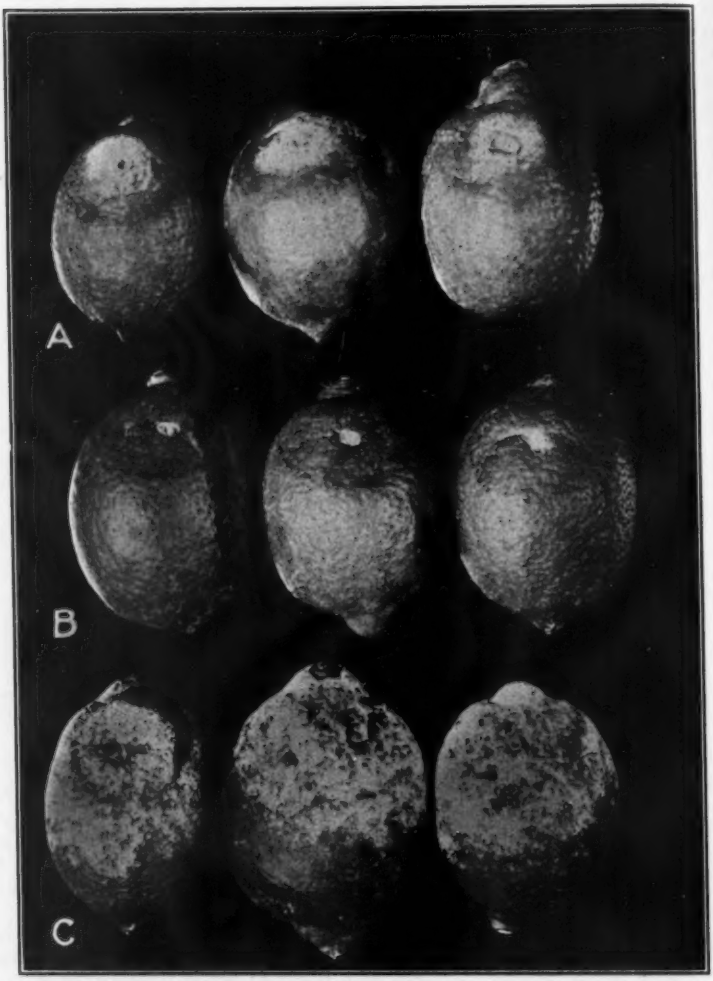

FIGURE 9.—Increased rate of decay in lemons produced by a mixed inoculation with *Penicillium italicum* and *P. digitatum* (C) as compared with inoculations of *P. digitatum* alone (A), or *P. italicum* alone (B); fruit held at 9.8° C. for eight days

a large number of the spores and at the same time to give to the remainder a sticky consistency and so prevent them from flying about during the isolation. In order to avoid contaminations three sterile scalpels were used in cutting, one for the advanced margin, another

for the place midway from the margin to the inoculation, and the last for the region under the inoculation. A small piece of decayed material in each test was transferred to glucose-potato agar.

TABLE 5.—*Rate of decay at different temperatures of lemons and oranges inoculated with two species of fungi separately and in combination*

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit kept at—						
		9.8° C.	14.1° C.	17.8° C.	21.2° C.	25.2° C.	27.6° C.	29.8° C.
Penicillium italicum	4	0	12	26	35	31	21	9
	6	10	26	48	62	61	37	10
P. digitatum	4	0	0	45	79	88	44	11
	6	11	33	120	* 150	* 150	* 150	12
P. italicum + P. digitatum	4	0	19	37	82	93	72	18
	6	22	48	86	* 150	* 150	* 150	24

ORANGES

P. italicum	4	0	12	26	34	40	35	40
	6	12	25	47	60	66	57	90
P. digitatum	4	0	22	48	73	92	95	77
	6	20	60	104	* 200	* 200	* 200	101
P italicum + P. digitatum	4	0	20	46	73	86	93	76
	6	15	55	106	* 200	* 200	* 200	93

* Entire circumference of fruit.

TABLE 6.—*Results of daily culture tests from decayed spots on fruits previously inoculated with a mixed spore suspension of Penicillium italicum and P. digitatum and kept at 21.5° C.*

LEMONS

Number of days after inoculation at which isolation tests were made	Place in the decayed spot from which culture tests were made		
	Advancing margin of decay	Midway from margin to wound	Under the inoculated wound
1	No growth	No growth	No growth.
2	P. digitatum	P. italicum + P. digitatum	P. italicum + P. digitatum.
	do	do	Do.
3	do	P. digitatum	Do.
	do	do	Do.
4	do	do	Do.
	do	P. italicum + P. digitatum	Do.
5	do	do	P. italicum.
	do	do	Do.

ORANGES

1	No growth	No growth	No growth.
2	P. digitatum	P. italicum + P. digitatum	P. italicum + P. digitatum.
	do	do	Do.
3	do	P. digitatum	Do.
	do	do	Do.
4	do	P. digitatum + P. italicum	Do.
	do	P. italicum + P. digitatum	Do.
5	do	P. digitatum	P. italicum.
	do	do	Do.

The results in Table 6 indicate that underneath the surface of the decay produced by a mixed inoculum of *Penicillium italicum* and *P. digitatum* at the favorable temperature of 21.5° C., *P. digitatum* will

be present at the advancing margins, either one or both will be present midway from the margin to the center, and both will live under the inoculated wound during the first few days, but after five days *P. digitatum* will be inhibited, leaving only *P. italicum* capable of being recovered.

COMBINED INOCULATIONS OF ASPERGILLUS NIGER, PENICILLIUM ITALICUM, AND P. DIGITATUM

TEMPERATURE RELATIONS OF ASPERGILLUS NIGER

This experiment was carried out by the methods previously described, at seven different constant temperatures with inoculations of five groups of lemons and five groups of oranges, as follows: (1) *Aspergillus niger*, (2) *A. niger* + *Pencillium italicum*, (3) *A. niger* + *P. digitatum*, (4) *A. niger* + *P. italicum* + *P. digitatum*, and (5) checks. The first and fifth groups contained each 35 lemons and 35 oranges divided into seven lots of 5 lemons and 5 oranges, and a lot was placed at each of the seven temperatures shown in Table 7. The second, third, and fourth groups contained 70 lemons and 70 oranges divided into lots of 10 lemons and 10 oranges and distributed like the first group.

During the eight days of the experiment no decay was evident from 9.6° to 19.3° C. either in lemons or oranges. After three days rotting had started at 23° and at 32.2°, developing rapidly at the latter temperature. Slight sporulation was nearly always contemporaneous with the beginning of decay. At high temperatures a pronounced white mycelial ring appeared in advance of the decay and in the center of this a black mass of spores was formed.

TEMPERATURE RELATIONS OF THE COMBINATIONS ASPERGILLUS NIGER + PENICILLIUM ITALICUM, A. NIGER + P. DIGITATUM, AND A. NIGER + P. ITALICUM + P. DIGITATUM

The rate of decay produced by the three combinations used in this group of experiments is shown in Table 7.

TABLE 7.—*Rate of decay at different temperatures of lemons and oranges inoculated with Aspergillus niger alone and in combination with other fungi*

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit kept at—						
		9.6° C.	14.7° C.	19.3° C.	23° C.	27.3° C.	30.1° C.	32.2° C.
A. niger	4	0	0	0	18	20	37	36
	6	0	0	0	32	42	59	60
A. niger + P. italicum	4	0	24	34	58	35	35	44
	6	16	42	60	66	50	53	63
A. niger + P. digitatum	4	0	44	78	113	103	36	49
	6	15	92	* 150	* 150	* 150	53	71
A. niger + P. italicum + P. digitatum	4	16	45	86	97	70	35	45
	6	50	93	* 150	* 150	* 150	54	57

ORANGES

A. niger	4	0	0	0	17	27	42	41
	6	0	0	0	27	42	65	78
A. niger + P. italicum	4	0	16	30	38	29	42	49
	6	16	32	42	63	43	67	77
A. niger + P. digitatum	4	0	38	64	89	91	46	55
	6	28	80	111	-------	115	64	80
A. niger + P. italicum + P. digitatum	4	16	41	66	89	102	47	50
	6	46	80	* 200	* 200	* 200	62	78

* Entire circumference of fruit.

The mixtures *Aspergillus niger* + *Penicillium digitatum* and *A. niger* + *P. italicum* + *P. digitatum*, showed higher rates of decay than the highest rate of any one of the fungi alone for both oranges and lemons at the two lowest temperatures, 9.6° and 14.7° C. (Compare Tables 5 and 7.) In lemons this increased rate extended up to 27.3°. A mixture of *A. niger* with *P. italicum* produced an increased rate at the lowest temperature, 9.6° in lemons and at both 9.6° and 14.7° in oranges. At higher temperatures than those mentioned the rates of the mixtures were equal to or slightly less than the highest rate of any component under the same conditions. The external characters of the combination *A. niger* + *P. italicum* were the same as those of pure *P. italicum* from 9.6° to 19.3°. *A. niger* occurred in the center for 5 to 20 mm., followed by *P. italicum*, which extended to the advancing margin at 23° to 27.3°. Only Aspergillus was found at 30° and above. No striking differences in the external appearance of decay on oranges and lemons were noted.

The external characters of the combination *Aspergillus niger* + *P. digitatum* were those of pure *P. digitatum* from 9.6° to 23° C., above which the *A. niger* appeared in the center over an area 5 to 22 mm. in diameter until a temperature of 30.1° and 32.2° was reached, when *A. niger* only was evident.

The external characters of the combination *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* were similar to those of the combination *P. italicum* + *P. digitatum* already described at temperatures of 9.6 to 23° C. At 27.3° *A. niger* appeared in the center of the decayed area, followed concentrically by *P. italicum* and then by *P. digitatum* up to the advancing margin. At 31.2° only *A. niger* was evident.

CULTURAL TESTS FROM TISSUES INSIDE THE FRUIT

At the end of the experiment fruits inoculated with the combination *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* from each of the seven chambers were used in isolation tests in order to find out what changes were taking place within the fruit in relation to those that showed on the surface. These tests were made from the "albedo" or inner part of the rind in the manner previously described in connection with *P. italicum* + *P. digitatum*. The results are shown in Table 8.

TABLE 8.—*Isolation culture tests from decayed spots on fruits inoculated with Aspergillus niger + Penicillium italicum + P. digitatum eight days previously*

LEMONS

Temperature (° C.)	Place in decayed spots from which culture tests were made		
	Advancing margin	Midway from margin to inoculation	Under the inoculated wound
9.6	P. italicum	P. italicum	P. italicum + A. niger.
14.7	P. digitatum	P. italicum + P digitatum	Do.
19.3	do	do	P. italicum.
23	do	do	A. niger.
27.3	do	P. digitatum + P. italicum	Do.
30.1	A niger + P. digitatum.	A. niger + P. digitatum + P. italicum.	Do.
32.2	A. niger	A. niger	A. niger + P. italicum.

TABLE 8.—*Isolation culture tests from decayed spots on fruits inoculated with Aspergillus niger + Penicillium italicum + P. digitatum eight days previously*—Con.

ORANGES

Temperature (° C.)	Place in decayed spots from which culture tests were made		
	Advancing margin	Midway from margin to inoculation	Under the inoculated wound
9.6	P. italicum + P. digitatum.	P. italicum + P. digitatum	P. italicum + A. niger.
14.7	P. digitatum	do	P. italicum + P. digitatum.
19.3	do	P. digitatum + P. italicum	Do.
23	do	P. digitatum	Do.
27.3	do	P. digitatum + P. italicum	A. niger.
30.1	A. niger + P. digitatum.	A. niger + P. italicum + P. digitatum.	A. niger + P. italicum.
32.2	A. niger	A. niger + P. italicum	Do.

In the advancing margin at the lowest temperature, 9.6° C., *Penicillium italicum* was found associated with the rotting of the fruit; from 14.7° to 27.3°, *P. digitatum;* at 30.1°, *Aspergillus niger* + *P. digitatum;* and at 32.2, *A. niger* alone was found. Since the spores of the various organisms were present in approximately the same amounts at the beginning, it is apparent that different temperatures exerted a selective influence, allowing one fungus in the mixture to become dominant at certain temperatures and permitting others to become dominant at other temperatures. It should also be noted that the fungi found under the surface did not always coincide with that which was seen sporulating on the surface above the place of isolation. At the completion of the experiment the fruit was taken out of the chambers and the lots were arranged in the order of the magnitude of the temperatures at which they had been kept. The effect produced by the combination *A. niger* + *P. italicum* + *P. digitatum* was very striking. The fruits from the lower-temperature chambers were predominantly blue in color due to spores of *P. italicum;* those from slightly higher temperatures showed a reduction of the blue color in the center with the green of *P. digitatum* spores predominating; those from still higher temperatures showed the black of *A. niger* spores in the center with the green of *P. digitatum* around them, and those at the highest temperatures showed only the black of *A. niger* spores.

COMBINED INOCULATIONS OF OOSPORA CITRI-AURANTII, PENICILLIUM ITALICUM, P. DIGITATUM, AND ASPERGILLUS NIGER

TEMPERATURE RELATIONS OF OOSPORA CITRI-AURANTII

Oospora citri-aurantii has, in comparison with many other rot fungi, a very wide range of temperature for decay. In lemons, as will be shown in the low-temperature experiments, it had during the six days of the experiment a minimum of about 7° to 8° C., an optimum of about 27° to 28°, and a maximum above 33°; in oranges it had a minimum of about 5° to 6°, an optimum of about 29° to 30°, and a maximum above 33° for the same time. In general the rot is soft in the beginning, but later becomes sunken and watery. In lemons the decay is much softer and more watery than in oranges. When completely rotted, the fruit is collapsed. Sporulation started

on lemons eight days after inoculation at a temperature of 23.5° and on oranges eight days after at 19.8°, and was absent on both lemons and oranges at 30° or above during the same period.

TEMPERATURE RELATIONS OF OOSPORA CITRI-AURANTII, PENICILLIUM ITALICUM P. DIGITATUM, AND ASPERGILLUS NIGER

An experiment was carried on at each of seven maintained temperatures with six lots of lemons and six lots of oranges as follows: (1) *Oospora citri-aurantii* alone, (2) *O. citri-aurantii* + *Penicillium italicum*, (3) *O. citri-aurantii* + *P. digitatum*, (4) *O. citri-aurantii* + *P. italicum* + *P. digitatum*, (5) *O. citri-aurantii* + *P. italicum* + *P. digitatum* + *Aspergillus niger*, (6) check. Each of these groups contained 35 lemons and 35 oranges divided into seven lots of 5 lemons and 5 oranges, and a lot was placed at each of the seven temperatures shown in Tables 9 and 10.

TABLE 9.—*Rate of decay at different temperatures of lemons and oranges inoculated with Oospora citri-aurantii alone and in combination with other fungi*

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit kept at—						
		11.5° C.	15.9° C.	19.8° C.	23.5° C.	27.2° C.	30.2° C.	31.9° C.
O. citri-aurantii	4	9	10	18	26	33	33	32
	6	16	20	43	49	59	57	46
O. citri-aurantii + P. italicum	4	14	15	30	40	38	39	42
	6	29	44	60	62	61	63	56
O. citri-aurantii + P. digitatum	4	15	29	67	108	107	64	42
	6	62	* 150	* 150	* 150	* 150	76	63
O. citri-aurantii + P. italicum + P. digitatum	4	17	48	97	118	107	101	43
	6	83	* 150	* 150	* 150	* 150	115	63

ORANGES

O. citri-aurantii	4	0	0	11	12	21	29	29
	6	0	10	27	29	45	55	52
O. citri-aurantii + P. italicum	4	12	21	29	38	22	41	45
	6	24	44	61	64	65	67	67
O. citri-aurantii + P. digitatum	4	11	32	58	83	95	87	37
	6	42	75	116	* 200	* 200	121	57
O. citri-aurantii + P. italicum + P. digitatum	4	14	32	65	90	100	96	35
	6	45	80	118	* 200	* 200	117	55

* Entire circumference of fruit.

In lemons, as shown in Table 9, the combination *Oospora citri-aurantii* + *Penicillium italicum* produced a more rapid decay than *O. citri-aurantii* or *P. italicum* alone (compare with Table 5), and the combination *O. citri-aurantii* + *P. digitatum* produced a decidedly more rapid growth than did *P. digitatum* or *O. citri-aurantii* alone; in fact at lower and intermediate temperatures it was equal to or greater than the sum of the rates of growth of *O. citri-aurantii* and *P. digitatum* when used alone.

In oranges the rate of decay caused by *Oospora citri-aurantii* + *Penicillium digitatum* was slightly greater at 11.5° to 23.5° C. than that caused by *P. digitatum* alone, but above these temperatures the growth was about the same except at the highest temperature (31.9°), where it was less. The combination *O. citri-aurantii* + *P. digitatum*

in the first two chambers (11.5° and 15.9°) gave an increment of decay somewhat greater than the sum of the increments given by *O. citri-aurantii* and *P. digitatum* alone, but from 19.8° to 31.9° it gave an increment constantly somewhat less than the sum. On the surface of lemons inoculated with *O. citri-aurantii* + *P. italicum* the spores of *P. italicum* were limited to a few millimeters at a temperature of 11.5°, but at 23.5° this rot had reached a size of 25 mm.;

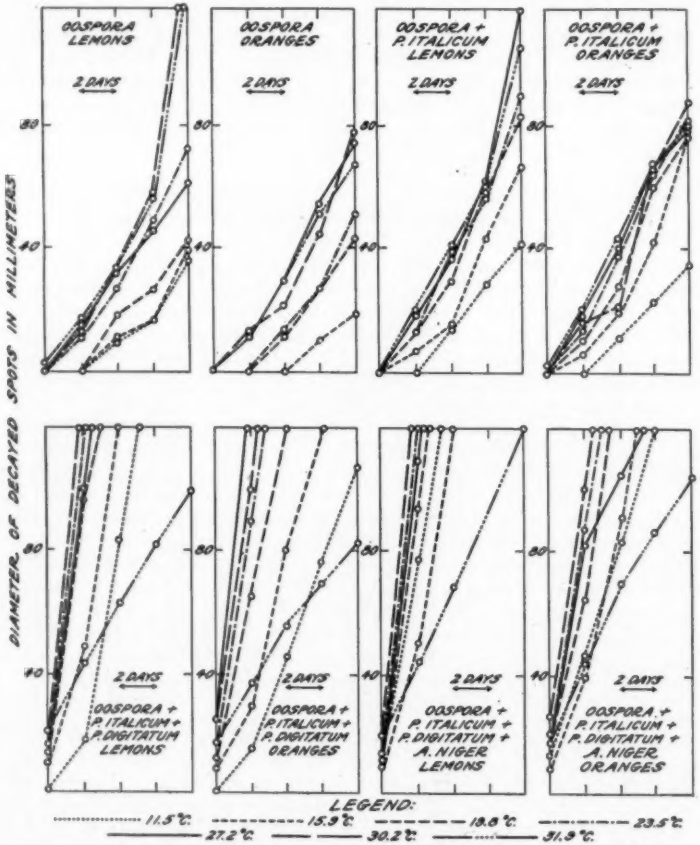

FIGURE 10.—Diameter of decayed areas on lemons and oranges produced by inoculations with *Oospora citri-aurantii* alone and mixed with other fungi; fruits were held at temperatures indicated for an 8-day period

above 23.5° only spores of *O. citri-aurantii* were seen. In all cases *O. citri-aurantii* was growing on the advancing margin of the decay. In oranges a similar manifestation was noted, with the difference that the spores of *P. italicum* were still present at 30°.

On the surface of the decayed areas resulting from inoculating oranges with *Oospora citri-aurantii* + *Penicillium digitatum*, only the mycelium of *P. digitatum* was evident at temperatures of 11.5° and

15.9° C. From 19.8° to 31.9° the spores of *O. citri-aurantii* were in the center of the area, and at 27.2° this rot had reached a diameter of 15 mm.

The combination in all cases resulted in *P. digitatum* actively growing in the advancing margin of the rot.

When lemons were inoculated with *Oospora citri-aurantii* + *Penicillium digitatum* oospora occurred at temperatures of 15.9° to 31.9° C. At 15.9° it occupied the center of the decayed area, surrounded by *P. digitatum*, which extended up to the margin. The area covered by *O. citri-aurantii* continued to increase at higher temperatures, until at 30.2° and 31.9° it had so enlarged as to occupy the entire decayed surface. The rot was watery and much softer than that produced by *P. digitatum* alone.

The growth of *Oospora citri-aurantii* + *Penicillium italicum* + *P. digitatum* may be compared to that of *P. italicum* + *P. digitatum*. (Table 9 and Table 5.) In lemons *O. citri-aurantii* + *P. italicum* + *P. digitatum* gave decidedly higher rates of growth than did *P. italicum* + *P. digitatum*, the rate being even higher than the sum of *O. citri-aurantii* alone plus the combination *P. italicum* + *P. digitatum*. In oranges the rate of decay was quite similar to that in lemons, except that at the two highest temperatures the growth of *O. citri-aurantii* + *P. italicum* + *P. digitatum* was somewhat less than the sum of the increment of *O. citri-aurantii* alone plus the combination *P. italicum* + *P. digitatum*. The combination of the three organisms produced a reddish discoloration underlying the zone of sporulation of *P. italicum* and other effects similar to those previously described for *P. italicum* + *P. digitatum*, with the following exceptions: *O. citri-aurantii* showed on the surface at the middle of the decayed area at 27.2° C. in both lemons and oranges and was the only fungus that showed at 31.9° C.

The addition of *Oospora citri-aurantii* to *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* appeared to increase the rate of decay on lemons but had no marked effect on oranges. (Table 10.) Figure 10 shows the rates of decay at different temperatures when *Oospora citri-aurantii* and the preceding mixtures were used.

TABLE 10.—*Rate of decay at different temperatures of lemons and oranges inoculated with Oospora citri-aurantii, Aspergillus niger, Penicillium italicum, and P. digitatum in two combinations*

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots in fruit started at—						
		9.6° C.	14.7° C.	19.3° C.	23° C.	27.3° C.	30.1° C.	32.2° C.
A. niger + P. italicum + P. digitatum	4	16	45	86	97	70	35	45
	6	50	93	*150	*150	*150	54	57
O. citri-aurantii + A. niger + P. italicum + P. digitatum	4	16	49	93	118	110	109	43
	6	77	*150	*150	*150	*150	*150	68

ORANGES

A. niger + P. italicum + P. digitatum	4	13	41	66	89	102	47	50
	6	31	80	*200	*200	*200	62	78
O. citri-aurantii + A. niger + P. italicum + P. digitatum	4	16	38	64	87	101	82	43
	6	46	91	117	*200	*200	105	60

* Entire circumference of fruit.

TABLE 11.—*Isolation tests from decayed spots in fruits inoculated eight days previously with Oospora citri-aurantii + Penicillium italicum + P. digitatum + Aspergillus niger*

LEMONS

Temperature (0° C.)	Place in decayed spot from which test was made		
	Advancing margin	Midway from margin to inoculation	Under the inoculation
11.5	O. citri-aurantii + P. italicum.	O. citri-aurantii + P. italicum	P. italicum.
15.9	O. citri-aurantii + P. digitatum + P. italicum.	do	O. citri-aurantii + P. italicum.
19.8	O. citri-aurantii + P. digitatum.	do	O. citri-aurantii.
23.0	P. digitatum	O. citri-aurantii	Do.
27.2	O. citri-aurantii	do	Do.
30.2	do	do	O. citri-aurantii + A. niger.
32.2	do	do	O. citri-aurantii.

ORANGES

11.5	O. citri-aurantii + P. italicum.	O. citri-aurantii + P. italicum	O. citri-aurantii + P. italicum.
15.9	O. citri-aurantii + P. digitatum + P. italicum.	O. citri-aurantii + P. digitatum + P. italicum.	Do.
19.8	P. digitatum + O. citri-aurantii.	P. italicum + O. citri-aurantii	P. italicum + O. citri-aurantii.
23.0	P. digitatum	do	Do.
27.2	do	do	Do.
30.2	O. citri-aurantii	O. citri-aurantii + A. niger	A. niger + O. citri-aurantii.
32.2	A. niger	A. niger	A. niger.

CULTURAL TESTS FROM TISSUES INSIDE THE FRUIT

At the end of the experiment some fruits from the combination of four organisms just mentioned were used for isolation purposes. The results of these experiments are shown in Table 11. In the lemons at high temperatures *Oospora citri-aurantii* was dominant. This may have been due to the fact that *O. citri-aurantii* attacks lemons more readily than oranges. In oranges *Aspergillus niger* was dominant. In the advancing margin of decay *O. citri-aurantii* was more often present in lemons than in oranges, having been reisolated six times out of seven in lemons and four times out of seven in oranges. Midway from margin to inoculation, *O. citri-aurantii* was found at all temperatures in lemons and at all except the highest in oranges. Under the inoculated wound in lemons *O. citri-aurantii* was found at all except the lowest temperatures and in oranges it was found at all except the highest temperature. *O. citri-aurantii* therefore appeared to dominate in lemons at high temperatures and *A. niger* in oranges at the same temperatures. The green mold, *P. digitatum*, was found only under the advancing margin and only at intermediate temperatures from 15.9° to 27.2° C. The blue mold, *P. italicum*, was found in all isolations in lemons and oranges at 11.5° and 15.9°, but above this was present more often in oranges than in lemons.

COMBINED INOCULATIONS OF BOTRYTIS CINEREA, PENICILLIUM ITALICUM, AND P. DIGITATUM AND OF B. CINEREA, P. ITALICUM, P. DIGITATUM, AND ASPERGILLUS NIGER

TEMPERATURE RELATIONS OF BOTRYTIS CINEREA

In preliminary tests with *Botrytis cinerea* alone the range of temperature for decay on lemons appeared to be greater than on oranges, probably because the former is a more natural host in the field. In 6 days the range for lemons was from about 2° to 26.8° C. (with an optimum about 22.5°) and for oranges from about 3° to 22.5° (with an optimum about 18.5°) under the same conditions. In both oranges and lemons the spores appeared first at 22.5° in 6 to 8 days after inoculation, at 14.5° in 12 days, and at 9.5° in 14 days. In oranges at 26.5° no spores were present even after 10 days; a small hard brown spot only was produced. No marked difference in color was observed at different temperatures.

TEMPERATURE RELATIONS OF BOTRYTIS CINEREA + PENICILLIUM ITALICUM + P. DIGITATUM, AND OF B. CINEREA + P. ITALICUM + P. DIGITATUM + ASPERGILLUS NIGER

Experiments were carried on at each of seven constant temperatures with four groups of lemons and four groups of oranges, with inocula as follows: (1) *Botrytis cinerea*, (2) *B. cinerea* + *Penicillium italicum* + *P. digitatum*, (3) *B. cinerea* + *P. italicum* + *P. digitatum* + *Aspergillus niger*, and (4) check. Each of the first three groups contained 70 lemons and 70 oranges divided into seven lots of 10 lemons and 10 oranges, and a lot was placed at each of the seven temperatures shown in Table 12. The fourth group contained 35 lemons and 35 oranges divided into seven lots of 5 lemons and 5 oranges, distributed as in the other groups.

TABLE 12.—*Rate of decay at different temperatures of lemons and oranges inoculated with Botrytis cinerea + Penicillium italicum + P. digitatum, and B. cinerea + P. italicum + P. digitatum + Aspergillus niger*

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit stored at—						
		9.5° C.	14.5° C.	18.5° C.	22.5° C.	26.8° C.	30.1° C.	31.1° C.
B. cinerea	4	0	19	20	22	16	0	0
	6	19	25	37	49	23	0	0
	8	29	46	62	88	33	0	0
	10	48	73	132	149	48	0	0
B. cinerea + P. italicum + P. digitatum	4	10	18	22	67	85	20	0
	6	22	43	64	• 160	• 160	28	0
B. cinerea + P. italicum + P. digitatum + A. niger	4	10	18	62	133	105	39	46
	6	19	53	• 160	• 160	• 160	67	81

ORANGES

B. cinerea	4	0	0	14	10	0	0	0
	6	9	12	22	13	0	0	0
	8	13	20	37	16	10	0	0
	10	24	31	47	18	10	0	0
B. cinerea + P. italicum + P. digitatum	4	0	10	31	66	89	61	0
	6	11	22	76	125	139	80	10
B. cinerea + P. italicum + P. digitatum + A. niger	4	0	12	32	62	71	34	37
	6	12	28	77	126	124	72	64

• Entire circumference of fruit.

The experiment was performed as previously indicated (p. 164). The growth of *Botrytis cinerea* alone and of the two combinations mentioned above is shown in Table 12. Observations on the decay due to the combination *B. cinerea* + *Penicillium italicum* + *P. digitatum* brought out several interesting points. In oranges at 9.5° C. the color of the decay was typical of *B. cinerea*, but the rot had a soft and watery form rather than the leathery pliability of Botrytis rot alone. At 14.5° C., some color due to Botrytis appeared after 4 days, followed by the blue spores of *P. italicum* in the center and mycelium of *P. digitatum* in the advancing margin after 8 days. At 18.5° *P. digitatum* was still more prominent, occupying 20 mm. on the advancing margin. At 22.5° *P. italicum* was reduced to a few millimeters around the inoculation wound, followed by Botrytis for 20 mm. and then *P. digitatum*. At 26.8° the Botrytis color was no longer seen on the outside, but when the fruit was cut through the inoculation wound, some of the brown color was found underneath. At 31.1° no growth of any of the three combined parasites was seen. The surface manifestations on lemons were similar to those on oranges. From about 22° to 30° *B. cinerea* + *P. italicum* + *P. digitatum* caused a higher rate of decay in lemons than *B. cinerea* alone or *P. italicum* + *P. digitatum*. (Compare Table 5 with Table 12.) At other temperatures the presence of *B. cinerea* seemed partially to inhibit the growth of *P. italicum* and *P. digitatum*. In oranges this inhibition extended to all temperatures.

From 9.5° to 26.8° C. no difference was found in the external growth of the combination *Botrytis cinerea* + *Penicillium italicum* + *P. digitatum* + *Aspergillus niger* as compared with that of the other combination shown in Table 12 until *Aspergillus niger* appeared. Four days after the inoculation, black spores of *A. niger* were present at 26.8° on oranges and lemons in the center of the decayed area, followed by *Penicillium italicum*, *B. cinerea*, and finally *P. digitatum* in concentric irregular rings. At 30.1° to 31.1° only A. *niger* was showing on the outside of the fruit.

Comparing the rate of decay of the above combination with that of *Botrytis cinerea* alone or with *Aspergillus niger* + *Penicillium italium* + *P. digitatum*, a decrease is noted from 9.5° to 18.5° C. and a pronounced increase from 22.5 to 31.1°. In oranges there is the same inhibition at low temperatures; that is from 9.5° to 26.8°, while at higher temperatures a slightly greater rate of decay occurs. The inhibition at lower temperatures seems to have been due to *B. cinerea*. At higher temperatures, favorable to active growth of *A. niger*, the rate of decay was increased by the presence of this fungus. As previously shown, *A. niger* also accelerated the rate of decay when used in combination with *P. italicum* and *P. digitatum*.

COMBINED INOCULATIONS OF ALTERNARIA CITRI, TRICHODERMA LIGNORUM, OOSPORA CITRI-AURANTII, BOTRYTIS CINEREA, PENICILLIUM ITALICUM, P. DIGITATUM AND ASPERGILLUS NIGER

The experiments to be reported here were carried out as previously indicated for each of the seven constant-temperature chambers with five groups of lemons and five groups of oranges, with inocula as follows: (1) *Alternaria citri*, (2) *Trichoderma lignorum*, (3) *Oospora citri-aurantii* + *T. lignorum* + *A. citri* + *Botrytis cinerea*, (4) *O. citri-*

aurantii + *T. lignorum* + *A. citri* + *B. cinerea* + *Pencillium italicum* + *P. digitatum* + *Aspergillus niger*, and (5) check. The first, second, and fifth groups each contained 35 lemons and 35 oranges, divided into seven lots of 5 lemons and 5 oranges, and a lot was placed at each of the seven temperatures shown in Table 13. The third and fourth groups contained 70 lemons and 70 oranges, divided into seven lots of 10 lemons and 10 oranges.

Previous to the use of the combination inoculations, the temperature relations of Alternaria and Trichoderma used singly were studied.

Alternaria citri will advance only slowly or not at all within perfectly sound immature fruit, but it readily invades mature fruit of low vitality. In lemons this experiment, as indicated in Table 13, showed in six days a minimum temperature for decay of 15.2° C., an optimum near 23.2° and a maximum near 27.6°. A longer period would have extended this range. In oranges no growth was observed during the short period of the experiment. Previous work (*1*) has shown that a much longer period would have been required.

Trichoderma lignorum produces a very characteristic form of decay, but one difficult to measure at the beginning because it is slow to show any difference in color or in softness until after it has proceeded for some time. The changes in color subsequently develop very rapidly. The fungus probably first invades the albedo and afterwards grows to and affects the surface over considerable areas all at once. In lemons it produced decay in six days only at 23.2°, 27.6°, and 30.1° C. (Table 13.) In oranges no decay took place in the 6 days of the experiment.

TABLE 13.—*Rate of decay at different temperatures of citrus fruits inoculated with combinations of fungi, in comparison with that of fruits inoculated with each fungus alone*

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots in fruit stored at temperatures indicated						
		11.5° C.	15.9° C.	19.8° C.	23.5° C.	27.2° C.	30.2° C.	31.9° C.
Oospora citri-aurantii	4	9	10	18	26	33	33	32
	6	16	20	43	49	59	57	46
		9.5° C.	14.5° C.	18.5° C.	22.5° C.	26.8° C.	30.1° C.	31.1° C.
Botrytis cinerea	4	0	13	20	22	16	0	0
	6	19	25	37	49	23	0	0
		9.7° C.	15.2° C.	19.6° C.	23.2° C.	27.6° C.	30.1° C.	31.1° C.
Alternaria citri	4	0	0	0	0	0	0	0
	6	0	0	10	12	10	12	0
Trichoderma lignorum	4	0	0	0	0	0	0	0
	6	0	0	0	47	72	84	0
O. citri-aurantii + B. cinerea + A. citri + T. lignorum.	4	10	14	18	38	41	39	37
	6	14	27	35	63	82	88	53
		9.7° C.	14.7° C.	19.3° C.	23° C.	27.2° C.	30.1° C.	32.2° C.
Aspergillus niger + Penicillium italicum + P. digitatum.	4	0	45	86	97	70	35	35
	6	16	93	150	150	150	54	57
A. niger + P. italicum + P. digitatum + A. citri + T. lignorum + B. cinerea + O. citri-aurantii.	4	15	28	68	88	74	88	38
	6	26	100	* 150	* 150	* 150	133	61

* Entire circumference of fruit.

TABLE 13.—*Rate of decay at different temperatures of citrus fruits inoculated with combinations of fungi, in comparison with that of fruits inoculated with each fungus alone*—Continued

ORANGES

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots in fruit stored at temperatures indicated						
		11.5° C.	15.9° C.	19.8° C.	23.5° C.	27.3° C.	30.2° C.	31.0° C.
O. citri-aurantii	4	0	0	11	12	21	29	29
	6	0	10	27	29	45	55	52
		9.5° C.	14.5° C.	18.5° C.	22.5° C.	26.8° C.	30.1° C.	31.1° C.
B. cinerea	4	0	0	14	14	0	0	0
	6	9	12	22	22	13	0	0
O. citri-aurantii + B. cinerea + T. lignorum + A. citri [b].								
		9.7° C.	14.7° C.	19.3° C.	23° C.	27.3° C.	30.1° C.	32.2° C.
A. niger + P. italicum + P. digitatum	4	0	41	66	89	102	47	50
	6	13	80	[a] 200	[a] 200	[a] 200	62	78
A. niger + P. italicum + P. digitatum + T. lignorum + B. cinerea + A. citri + O. citri-aurantii.	4	9	23	43	64	72	27	23
	6	21	60	96	118	126	45	42

[a] Entire circumference of fruit.
[b] No decay from T. lignorum, A. citri or this combination in six days.

The combination of the four fungi, *Oospora citri-aurantii* + *Botrytis cinerea* + *Altenaria citri* + *Trichoderma lignorum*, in lemons (Table 13) shows an increase in rate of decay as compared to that of any of these fungi acting alone at a temperature about 23° C. and higher. The rate of growth of the combination of the seven fungi (Table 13) showed a slight increase over that of *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* only at the three lowest and the two highest temperatures.

In oranges the combination of the seven fungi (Table 13) showed a rate of decay less than that of *Aspergillus niger* + *Penicillium italicum* + *P. digitatum* except at 9.7° C. The external manifestations were quite similar to those noted in the experiment with *Botrytis cinerea* + *P. italicum* + *P. digitatum* + *A. niger* already described.

Isolation tests from decayed spots produced by a mixture of the seven fungi are shown in Table 14. Isolations were made from the albedo region under the surface. As may be seen from this table, *Oospora citri-aurantii* was isolated in every case except one in lemons, and it was the only fungus found at 32.2° C., while in oranges *O. citri-aurantii* was not isolated so often and was displaced by *Aspergillus niger* at 32.2°. In this respect this combination of seven fungi gave an isolation response similar to that of the combination *O. citri-aurantii* + *Penicillium italicum* + *P. digitatum* + *A. niger* of Table 11. *Trichoderma lignorum* was also isolated only under the inoculated wound in both oranges and lemons from 19.3° to 30.1°. *Botrytis cinerea* did not appear in any of the isolation tests.

TABLE 14.—*Isolation cultures from decayed spots produced by inoculations with a mixture of Aspergillus niger, Penicillium italicum, P. digitatum, Alternaria citri, Trichoderma lignorum, Botrytis cinerea, and Oospora citri-aurantii*

LEMONS

Temperature (° C.)	Advancing margin of decay	Place in decayed spots from which tests were made	
		Midway from margin rot to wound	Under the inoculated wound
19.3	O. citri-aurantii + Penicillium digitatum.	O. citri-aurantii + P. italicum	O. citri-aurantii + T. lignorum.
20.3	P. digitatum	O. citri-aurantii	A. citri + O. citri-aurantii.
27.3	O. citri-aurantii	do	T. lignorum + O. citri-aurantii.
30.1	do	do	T. lignorum + O. citri-aurantii + A. niger.
32.2	do	do	O. citri-aurantii.

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19.3	P. digitatum + O. citri-aurantii.	P. italicum + O. citri-aurantii	P. italicum + O. citri-aurantii + T. lignorum.
20.3	P. digitatum	do	T. lignorum + P. italicum + O. citri-aurantii.
27.3	do	O. citri-aurantii	P. digitatum + O. citri-aurantii + T. lignorum.
30.1	O. citri-aurantii	O. citri-aurantii + A. niger	T. lignorum + A. niger + O. citri-aurantii.
32.2	Aspergillus niger	A. niger	A. niger.

COMBINATION EXPERIMENTS AT LOW AND MEDIUM TEMPERATURES

Some of the more important organisms known to produce decay at low or medium temperatures were tested by inoculating fruits with them and placing the fruits in seven different temperature chambers ranging from 3° to 18.3° C. This experiment was begun on July 11, 1927.

Seven groups each containing 35 lemons and 35 oranges were used, with inocula as follows: (1) *Penicillium italicum*, (2) *P. digitatum*, (3) *Botrytis cinerea*, (4) *Oospora citri-aurantii*, (5) *Sclerotinia libertiana*, (6) *P. italicum* + *P. digitatum* + *B. cinerea* + *O. citri-aurantii* + *S. libertiana*, and (7) check. Each group was divided into seven lots of 5 lemons and 5 oranges, and a lot was placed at each of the seven temperatures shown in Table 15. Bits of mycelia instead of spores were used for inoculations with *S. libertiana*.

It was not feasible to combine the rates of decay in this experiment (Table 15) with those shown in Tables 5, 9, and 12 since it was necessary to use fruits of a different degree of maturity because of the difference in the season. The previous experiment was made in February and the last one in July. In most cases in oranges the rates of decay were higher in this experiment than in the earlier ones where a few of the same temperatures were again used. (Compare Tables 5, 9, and 12 with Table 15.) However, with lemons at some of the temperatures there was considerable agreement. The actual rate of decay in any one chamber should be considered of less significance than the differences in rates of decay in relation to temperature as a whole. (Table 15.)

TABLE 15.—*Rate of decay at low and medium temperatures of lemons and oranges inoculated with various fungi*

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit stored at—						
		3° C.	7.3° C.	10.2° C.	12.9° C.	16° C.	17.4° C.	18.3° C.
Penicillium italicum	4	0	0	11	20	29	37	41
	6	0	13	24	40	51	70	75
	8	0	26	45	70	73	98	103
P. digitatum	4	0	0	13	44	79	110	138
	6	0	20	42	123	151	150	150
	8	0	66	104	148	*160	*160	*160
Botrytis cinerea	4	0	0	0	10	14	18	16
	6	0	0	13	22	38	27	29
	8	0	12	28	46	72	*160	92
Oospora citri-aurantii	4	0	0	0	0	17	24	25
	6	0	0	10	22	37	44	48
	8	0	0	22	41	55	54	68
Sclerotinia libertiana	4	0	0	17	10	35	50	34
	6	0	0	22	25	81	122	87
	8	0	15	47	57	128	148	150
P. italicum + *P. digitatum* + *O. citri-aurantii* + *B. cinerea* + *S. libertiana*	4	0	0	10	17	59	94	136
	6	0	9	20	89	155	155	154
	8	0	20	83	150	*160	*160	*160

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P. italicum	4	0	0	13	21	32	33	30
	6	0	11	43	83	117	130	135
	8	0	17	69	113	114	158	160
B. cinerea	4	0	0	0	0	13	12	10
	6	0	0	0	10	15	18	14
	8	0	0	8	15	18	22	18
O. citri-aurantii	4	0	0	0	8	10	17	17
	6	0	16	20	14	15	41	30
	8	0	19	63	30	20	58	40
S. libertiana	4	0	0	0	0	15	10	12
	6	0	0	0	0	25	30	28
	8	0	0	0	0	56	77	*200
P. italicum + *B. cinerea* + *O. citri-aurantii* + *S. libertiana*	4	0	14	25	40	55	85	80
	6	0	16	46	75	99	136	136
	8	0	25	73	107	136	160	160

* Entire circumference of fruit.

The external manifestations of the fungi not in combination showed no marked differences from those already described in previous experiments except that spores of *Penicillium italicum* appeared at a temperature as low as 7.3° C. after six days. In the combination at 7.3° more *P. italicum* showed outside than did *P. digitatum;* at 10.2° and 12.9°. *P. italicum* occurred in the center followed by *Botrytis cinerea* and then by *P. digitatum* up to the advancing margin; at temperatures from 16° to 18° *B. cinerea* was displaced by *Oospora citri-aurantii*. *P. digitatum* was the only fungus isolated from the margin of decay in the fruit of four different chambers.

In lemons the combination of fungi seemed to produce a decided decrease in rate of decay as compared with the fungus producing the highest rate of decay when acting alone. In oranges the combination seemed to hasten slightly the beginning of decay at low temperatures (7° to 10°), but from 13° to 18° it produced about the same rate of decay as did *Penicillium italicum* alone.

COMBINATIONS WITH INOCULUM OF MYCELIUM

An experiment was carried out at each of the seven maintained temperatures with six groups of lemons and six groups of oranges, with inocula as follows: (1) *Diplodia natalensis*, (2) *Dothiorella ribis*, (3) *Phomopsis californica*, (4) *Pythiacystis citrophthora*, (5) *Sclerotinia libertiana*, and (6) a mixture of the five fungi just mentioned. Each group contained 35 lemons and 35 oranges divided into seven lots of 5 lemons and 5 oranges, and a lot placed at each of the temperatures shown in Table 16.

This experiment was performed as previously indicated except for the manner of preparing the inoculum. The mycelia of fungi which fruit less rapidly in culture were transferred to tubes of sterile water and crushed out in very small pieces. A tube of melted glucose-potato agar at 41° C. was transferred to the crushed mycelium and shaken constantly while it was solidifying. A fairly uniform distribution of the inoculum resulted. A small amount of sterile water was added until a gelatinous mixture was obtained. The wound in the fruit made as in previous experiments was filled with this mixture of mycelium by means of a small spatula. The results are given in Table 16.

TABLE 16.—*Rate of decay at different temperatures of lemons and oranges inoculated with various fungi alone and in combination*

LEMONS

Inoculum	Days after inoculation	Diameter in millimeters of decayed spots on fruit stored at—						
		6.5° C.	13.1° C.	18.2°C.	22.8°C.	27.6°C.	30.7°C.	32.8°C.
Diplodia natalensis	4	0	0	21	59	80	155	116
	6	0	16	54	59	154	160	159
	8	0	24	54	155	* 160	* 160	* 160
Dothiorella ribis	4	0	0	0	11	13	10	8
	6	0	9	16	15	14	11	8
	8	0	9	32	32	18	13	8
Phomopsis californica	4	0	0	13	14	0	8	0
	6	0	20	35	34	9	8	0
	8	0	24	50	60	10	8	0
Pythiacystis citrophthora	4	10	32	55	69	89	43	0
	6	15	57	88	130	139	44	0
	8	25	80	118	155	* 160	60	0
Sclerotinia libertiana	4	0	0	32	38	15	10	0
	6	19	37	63	80	60	14	0
	8	23	83	105	120	105	18	0
Mixture of above fungi	4	0	19	38	78	155	155	155
	6	0	22	97	155	* 160	* 160	* 160
	8	0	35	155	* 160	* 160	* 160	* 160

ORANGES

D. natalensis	4	0	0	11	40	74	101	96
	6	0	0	35	99	126	167	152
	8	0	0	60	* 200	* 200	* 200	* 200
D. ribis	4	0	0	0	10	11	10	11
	6	0	0	12	14	16	12	11
	8	0	0	12	23	22	13	13
P. californica [1]								
P. citrophthora	4	0	0	23	41	51	26	0
	6	0	25	54	79	76	27	0
	8	0	32	74	101	114	28	0
S. libertiana	4	0	0	15	15	0	0	0
	6	0	0	55	80	0	0	0
	8	0	0	85	130	0	0	0
Mixture of above fungi	4	0	0	33	67	74	* 200	117
	6	0	0	39	110	106	* 200	* 200
	8	0	0	62	* 200	* 200	* 200	* 200

[1] No decay in 8 days.

* Entire circumference of fruit.

Both in lemons and in oranges the combined inoculations resulted in the complete dominance of the external color and odor characteristic of *Diplodia natalensis*. The rapidity of decay of lemons due to the mixture was less than that of the most rapid organism acting alone at 6.5° and 13.1° C., but the rapidity of decay due to the mixture was greater at higher temperatures. In oranges the combination produced an inhibition at 6.5° and 13.1°, an increase at 30.7° and 32.8°, but showed a decay about equal to that of *D. natalensis* at intermediate temperatures. Using the method previously indicated, the writers made 40 isolations from the combined inoculated fruits, taking the tissue from under the advancing margin, midway from margin to center, and under the inoculated wound. In 38 cases the cultural tests showed the presence of *D. natalensis* alone.

TABLE 17.—*Average percentage of the circumference of lemons rotted six and eight days after inoculation with various fungi when kept at temperatures indicated*

Inoculum	Days after inoculation	Percentage of circumference rotted when stored at—															
		3° C.	6° C.	7° C.	10° C.	13° C.	16° C.	18° C.	20° C.	23° C.	25° C.	27° C.	28° C.	30° C.	31° C.	32° C.	33° C.
Aspergillus niger	6				0		0	0		21.0		26.8		37.7		38	
	8				0		0	0		26.8		35.2		100.0		100.0	
Diplodia natalensis	6		0			10.2		34.5		100.0			100.0		100.0		100.0
	8		0			15.3		90.7		100.0			100.0		100.0		100.0
Oospora citri-aurantii	6	0		0	10.2		12.8		16.6	31.3		37.7		42.8		29.4	
	8	0		0	18.5		24.9		27.5	46.7		100		100.0		39.6	
Trichoderma lignorum	6				0		0		0	27.2			41.7		48.7		0
	8				0		6.3		20.8	64.3			69		87.0		20.3
Pythiacystis citrophthora	6		9.6			36.5		56.3		93.9			93.9		28.1		0
	8		22.4			51.2		75.5		100.0			100.0		38.4		0
Penicillium digitatum	6	0		12.8	26.8	78.0	100.0	100.0			100		100.0	0			
	8	0		42.2	66.5	100.0								0			
P. italicum	6	0		8.3	15.3	25.6	32.6	48.0			39		23.8	0			
	8	0		16.6	28.8	44.8	46.7	65.9			53.1		40.3	0			
Phomopsis californica	6		0			12.8		22.4		21.7			5.7		0		0
	8		0			16.0		32.0		38.4			5.7		0		0
Dothiorella ribis	6		0			5.7		10.2		9.6			7.9		7.0		0
	8		0			5.7		20.4		20.4			11.5		8.3		0
Sclerotinia libertiana	6	0	12.1			23.8		40.3		51.2			38.4		6.9		0
	8	0	14.7			27.5		67.2		76.8			67.2		11.5		0
Botrytis cinerea	6	0	0		12.1	16.0		23.6		31.3			14.7		0		0
	8	0	8.2		18.5	29.4		39.6		56.3			20.1		0		0

TABLE 18.—*Average percentage of the circumference of oranges rotted six and eight days after inoculation with various fungi when kept at temperatures indicated* [a]

Inoculum	Days after inoculation	Percentage of circumference rotted when stored at—																		
		3° C.	6° C.	7° C.	10° C.	13° C.	15° C.	16° C.	18° C.	19° C.	20° C.	21° C.	23° C.	25° C.	27° C.	28° C.	30° C.	31° C.	32° C.	33° C.
Aspergillus niger	6				0		0			0			11.6		24.3		37.7		45.2	
	8				0		0			8.7			20.8		37.7		100.0		100.0	
Diplodia natalensis	6		0			0			20.3				57.4			73.0		100.0		88.1
	8		0			0			34.8				100.0			100.0				100.0
Oospora citri-aurantii	6	0		9.2		8.7		7.5			15.6		16.8		26.1				30.1	
	8	0		11.0		19.2		11.6			25.5		30.1		45.8				40.0	
Trichoderma lignorum	6				0		0				0		0			0		0		0
	8				0		0				0		23.2			0		0		0
Pythiacystis citrophthora	6		0			14.5			31.3				45.8			43.4		15.6		0
	8		0			18.5			42.9				58.5			66.1		16.8		0
Penicillium digitatum	6	0		6.3	24.9			41.1				100.0		100.0		100.0	60.6			
	8	0		9.8	40.5			69.8									66.1			
P. italicum	6	0		7.5	14.5			29.0				34.8		38.2		20.3	44.6			
	8	0		12.7	21.4			41.1				50.4		49.8		47.5	57			
Dothiorella ribis	6		0			0			6.9				8.1			9.1		6.9		0
	8		0			0			6.9				13.3			12.7		6.9		0
Sclerotinia libertiana	6	0				0			35.2				51.2							14.0
	8	0				0			54.4				83.2							22.4
Botrytis cinerea	6	0			5.2			8.7	12.7				7.5			0		0		0
	8	0			7.5			10.4	21.4				9.2			0		0		0

[a] No growth after 8 days after inoculation with *Phomopsis californica*.

COMPARISON OF RATES OF DECAY FOR ALL THE FUNGI USED

Tables 17 and 18 show the decay in terms of percentage of the circumference of the fruit rotted at the temperatures and times indicated. The fungi are arranged in a manner to show those that produce decay most readily at higher temperatures as compared with those that produce decay most readily at lower temperatures. The fungi producing decay most rapidly at the higher temperatures, 30° to 33° C., on lemons are *Aspergillus niger*, *Diplodia natalensis*, *Oospora citri-aurantii*, and *Trichoderma lignorum*. The fungi producing decay in eight days at the low temperature of 7° or below are *Pythiacystis citrophthora*, *Sclerotinia libertiana*, *Botrytis cinerea*, *Penicillium digitatum*, and *P. italicum*. All the fungi produce decay readily at the intermediate temperatures.

DISCUSSION AND CONCLUSIONS

The results of these experiments as a whole, although somewhat confusing in certain particulars, appear to justify certain rather definite conclusions.

Some of the striking features to be noted are: (1) The selective effect of temperature, in many cases enabling one organism in a mixture to dominate the others in producing decay; (2) the depressing or accelerating effect of a given mixture on rate of decay as compared with that produced by the most rapidly growing organisms of the mixture when used alone; (3) the influence of given mixtures of organisms on the color and consistency of decay; and (4) the differences in temperature range, and in optimum, maximum, and minimum temperatures of the various organisms for decay during a given time.

The selective effect of temperature was strikingly shown in the mixture of *Aspergillus niger*, *Penicillium digitatum*, and *P. italicum* where Aspergillus dominated all the others at the higher temperatures and *P. italicum* at the lower, with graduating mixtures at intermediate temperatures. This selective effect in most cases (not all) appeared to be related to the proximity of the given temperature to the range for optimum growth of the dominant fungus when acting alone. *A. niger* is known to grow well at temperatures above which the two species of Penicillium make only a slow growth. *P. italicum*, on the other hand, grows better at lower temperatures than *P. digitatum*, and at such temperatures *A. niger* makes a very feeble growth.

The selective effect also appears to be influenced by the resistance or susceptibility of the host as shown in the mixture *Penicillium digitatum* + *P. italicum* + *Oospora citri-aurantii* + *Aspergillus niger*, where *O. citri-aurantii* dominated at the highest temperature in lemons and *A. niger* in oranges. While lemons and oranges show very little difference in susceptibility to *A. niger*, lemons are much more susceptible to *O. citri-aurantii* than are oranges. In the mixture, therefore, *O. citri-aurantii* was able to dominate *A. niger* in lemons, but not in oranges at the same range of temperature.

The increase or decrease in rate of decay produced by mixtures of fungi as compared to the rate of decay caused by the most rapid organisms alone was marked in some cases, but in many others the

rate of decay was not greatly changed. This increase or decrease varied not only with temperature, but with different combinations of organisms. The mechanism of this depression or acceleration of the mixture is probably related to the combinations of enzymes that are present and their action in making food materials for growth available, or in producing inhibiting substances. The specific food requirements of the respective fungi and the competition which these imply are probably of prime importance in attempting an explanation of the behavior of the fungi in mixtures. Further work on this phase of decay is greatly needed. Qualitative and quantitative study of the enzymes of the fungi is suggested. The most striking example of increase in decay due to the combined effect of organisms is the combination *Oospora citri-aurantii* and *Penicillium digitatum*, where the combined inoculation showed a rate of decay as great as or even greater than the sum of the rates of *O. citri-aurantii* and *P. digitatum* when used separately. Similar effects, though less pronounced, due to other combinations were also evident, such as certain mixtures containing *Diplodia natalensis*. Depression in rate of decay due to mixed inoculations was not so striking, but was evident in a number of cases. The rate of decay caused by a mixture of *P. digitatum* and *P. italicum* was less than that of *P. digitatum* alone except at the highest and lowest temperatures. The presence of *Botrytis cinerea* in certain mixtures also had a retarding effect on decay as compared to the rate of the most rapid organism of the mixture. The influence of mixtures of organisms on the type of decay was often very noticeable. Increase in softness or in ease of puncturing or pliability was noted in mixtures as compared to decay caused by any one component of the mixture acting alone. Color changes on the surface or in the interior were also noted, as for example, the pinkish discoloration produced by a mixed infection of *P. italicum* and *P. digitatum* at certain temperatures. The experiments served to confirm previous observations which indicated that this characteristic may be used to determine whether or not a mixture of the two organisms is present in decayed fruits.

The results also serve to point out in a general way which of the various fruit-decay organisms are most important at the different ranges of temperature in storage. A low-temperature group as contrasted with a high-temperature group was evident. At an intermediate range of temperature some organisms from both the low-temperature and high-temperature groups are seen to be important. It should be pointed out that, if given sufficient time, many of these organisms may produce some decay over much wider ranges than those indicated in these short-time experiments.

The results of this investigation have served to suggest a partial explanation for certain decay phenomena in field, packing houses, and during transit, that were not previously well understood. Mixtures of spores of different organisms are more likely to be present under the usual conditions of handling the fruit than spores of a single organism. The results obtained from mixed inocula, therefore, together with the results from single inoculations, give a better basis for the interpretation of what goes on under commercial conditions.

SUMMARY

Some of the conditions which might cause variations in the rate of decay of citrus fruits, aside from mixture of organisms and temperature, were first considered.

It was determined that a variation in the number of *Penicillium italicum* spores between approximately 125,000 and 2,000,000 per cubic centimeter of inocula made little difference in the rate of decay.

At an air humidity of about 43° and an air temperature of 22.2° C. the rate of decay of citrus fruits inoculated with *Penicillium italicum* was the same whether the inoculum was inserted 3, 6, or 9 days after they were picked. When the fruits were inoculated soon after they were picked, the rate of decay was slightly greater.

It was found that seven lots of mature lemons, each held for two weeks at a different temperature, ranging from 9.8° to 31° C., and then inoculated with *Penicillium italicum* showed no difference in rate of decay due to differences in previous exposure.

Lemon fruits held at temperatures alternating from 9.8° to 27.1° C. every 12 hours showed average rates of decay not far from those of fruits held at a constant intermediate temperature.

An increasing rate of decay related to changes in color of lemons from deep green to deep yellow was found. This increasing rate of decay also corresponded to a decreasing resistance of the rind to a pressure necessary to puncture it.

When inocula containing certain mixtures of spores were used the rate of decay greatly increased; when inocula containing certain other mixtures were used the rate was lower than when any one of the components of the mixture was used alone; and when inocula containing still other mixtures were used the rate approximated that of the most rapid component acting alone. With most mixtures temperature had an important influence on decrease or increase in rate.

A mixed inoculum containing spores of *Penicillium italicum* and *P. digitatum* gave increased rates of decay on lemons at the higher and lower ends of the temperature range. The mixture also produced a reddish discoloration of the decayed tissue.

A mixed inoculum containing *Aspergillus niger*, *Penicillium italicum*, and *P. digitatum* produced at the lower temperatures higher rates of decay than the highest rate of any one of the fungi alone.

Oospora citri-aurantii in mixtures with *Penicillium italicum* and *P. digitatum* caused greatly accelerated rates of decay, especially on lemons, where at low and intermediate temperatures the rate was more than the sum of the rates produced by the fungi alone.

Botrytis cinerea in mixtures with *Penicillium italicum* and *P. digitatum* appeared to have a depressing effect on the rate of decay.

In other mixtures Botrytis cinerea appeared as a rule to have a depressing effect at low and intermediate temperatures, *Aspergillus niger* to have an accelerating effect at intermediate and high temperatures, and *Oospora citri-aurantii* to have an accelerating effect at nearly all temperatures.

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COTTON ROOT-ROT INVESTIGATIONS IN ARIZONA[1]

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INTRODUCTION

Studies have been conducted during recent years on the cotton root-rot disease at different places in the Southwestern States. These have contributed new information on the habits of the fungus,[2] its manner of spreading through the soil, its recurrence from year to year, its fruiting behavior, factors influencing its severity, and treatments that check its advance or effect its partial control. During the seasons 1925, 1926, and 1927 further evidence was obtained at the United States Field Station at Sacaton, Ariz., that certain chemical disinfectants and organic manures are effective in reducing the injury and that the fungus is more destructive and more persistent in recurrence at the margins of areas where new territory is being invaded. The behavior of the fungus in cultures on different media, its ability to grow on dead roots, and the relationship of dead roots in carrying over the disease in the soil were also studied. The results of these experiments and observations are reported and discussed in this paper.

USE OF CHEMICAL DISINFECTANTS

The possibility of controlling root rot by the use of disinfectants applied to the soil (fig. 1) has occurred to several investigators, but the results of experiments have not indicated that control by such measures can be made practicable.

The efficiency of formaldehyde for eradicating the disease for small, isolated spots has been reported by the senior writer in previous publications (*1*, *2*).[3] Recognizing the advantages afforded under irrigation for applying solutions that should be expected to reach the fungus in all parts of the root zone, the senior writer conducted experiments in 1923 and 1924 irrigating badly infected areas with solutions of formaldehyde. The results of these tests (*2*) were sufficiently promising to justify further study.

When the tests were continued in 1927 it had become known from previous study that the disease is likely to show more injuries in areas that were only recently involved than in areas that have shown the disease in varying degrees for several years. For this reason plots were selected for treatment on the outside margin of a root-rot area where there had been a recent invasion. A circular area of disease had extended into plot C2–15 about 1919 and by 1925 had crossed into C2–14, a distance of 53 feet. The diseased area in these two plots was measured and charted in 1926. (Fig. 2.) In the spring of 1927 this area, together with the marginal area 4 feet beyond

[1] Received for publication Sept. 21, 1928; issued August, 1929.
[2] *Phymatotrichum omnivorum* (Shear) Duggar.
[3] Reference is made by italic numbers in parentheses to "Literature cited," p. 221.

the arc of the active disease, was checked off into basins about 8 by 8 feet. The basined area on plot C2–14, which included 452 square feet, was treated with carbon bisulphide, applied with the irrigation

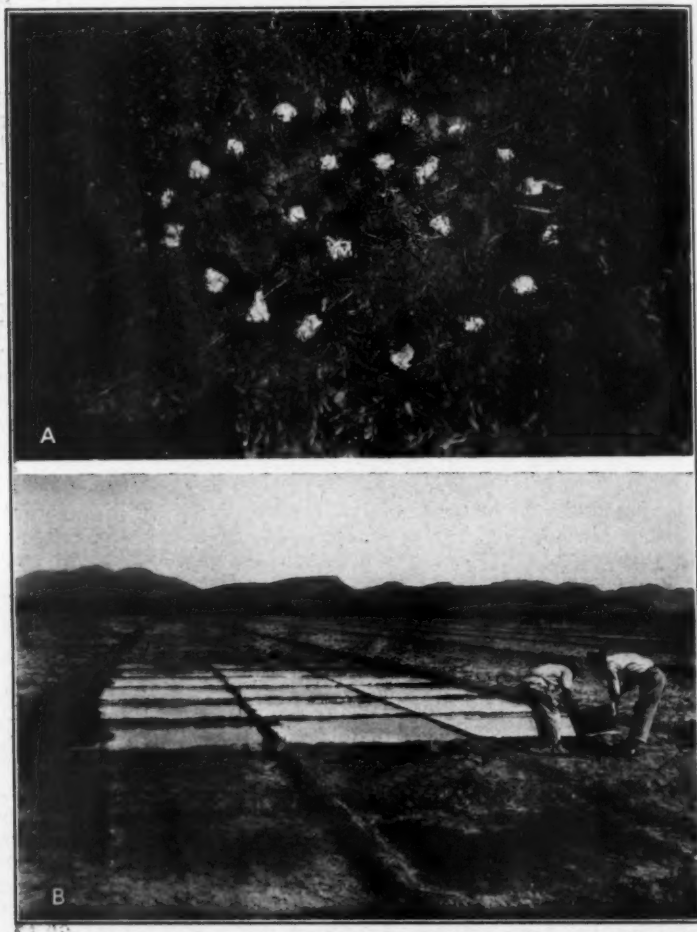

FIGURE 1.—A, Attempt to exterminate small isolated spots of root-rot disease in an alfalfa field by perforating the soil around the dead plants with holes and repeatedly filling these with formaldehyde. Only two plants shown in the inner circle of plugged holes had died when the treatment was made, and the disease in this case was arrested, but many similar trials were unsuccessful in preventing further spread through the soil. B, Method of applying disinfectants to infected areas before planting cotton in the spring. Formalin (1 per cent) and cresylic acid (2 per cent) were found to be very effective in preventing the recurrence of the disease where it had extended into new areas

water, 1 part to 100 at the rate of 3 acre-inches. On plot C2–15 an area of 1,903 square feet was basined, and 3 acre-inches of a 2 per cent solution of cresylic acid was applied to 1,080 square feet. To

the remaining 823 square feet a solution of formaldehyde (1–100) was applied, likewise at the rate of 3 acre-inches. (Fig. 1, B.)

The disinfectants were applied on April 22, just after a stand of Pima cotton seedlings had broken through the ground. All of the cotton seedlings were killed by the different solutions, and where the cresylic acid was applied Bermuda grass which had been turned under without being killed was apparently exterminated. The cotton was replanted on April 28, and the new seedlings appeared to be little affected by the disinfection of the soil. Germination, however, was a little slower where the cresylic acid had been applied.

It was apparent early in the season that the carbon-bisulphide treatment on plot C2–14 had not reduced the root rot to any great extent. Several centers of infection appeared in July and August,

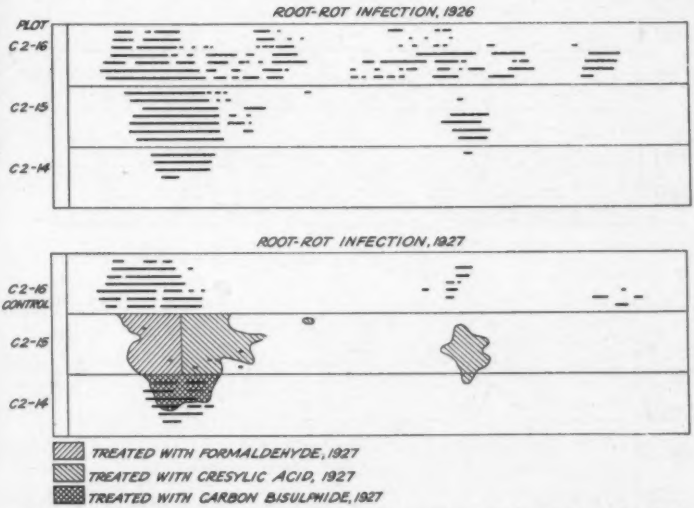

FIGURE 2.—Effects of formaldehyde and cresylic acid on areas recently invaded by the root-rot fungus in plot C2-15. The carbon-bisulphide treatment on plot C2-14 apparently had no effect on the fungus. Infection shown by heavy lines

and by the end of the season the infection was more extensive than in the year previous. On plot C2–15, however, the areas treated with cresylic-acid and formaldehyde solutions showed no infection until late in the season, and only five plants were dead when inspection was made on October 29.

Diagrams showing the extent of the disease in 1926 and 1927 on these two plots and on C2–16, used for control, are shown in Figure 2. Although the extent of infection on control plot C2–16 was less in 1927 than in the year previous, it will be noted that the three principal centers of infection still persisted, whereas in plot C2–15 one of the two principal spots of infection disappeared entirely, and the other showed no evidence of the disease until late in the season, when a few small spots appeared and six or eight plants were killed before the first frost occurred.

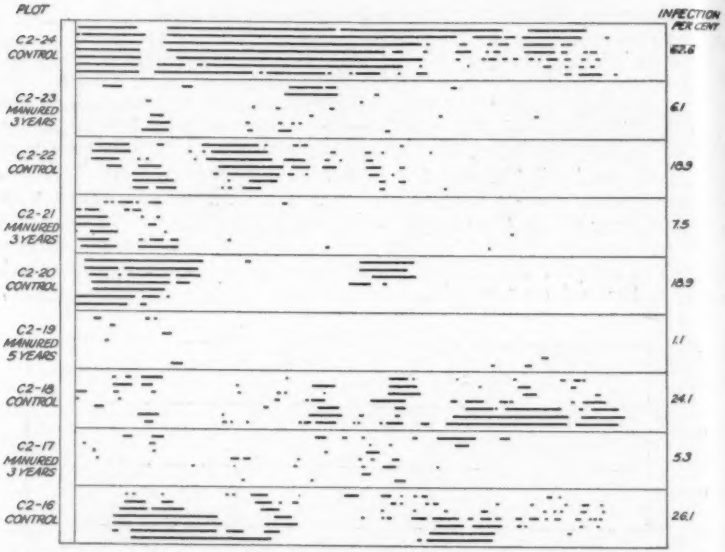

FIGURE 3.—Area infected by root rot (heavy lines) in quarter-acre experimental plots at the end of the season of 1925. Plots C2-17, C2-21, and C2-23 had received applications of organic materials for three years and plot C2-19 for five years

FIGURE 4.—Area infected by root rot (heavy lines) in quarter-acre experimental plots at the end of the season of 1926. Plots C2-17, C2-21, and C2-23 had received applications of organic materials for four years and plot C2-19 for six years

CONTROL EXPERIMENTS WITH ORGANIC MANURES

The effects of farmyard manure and rotted alfalfa on the occurrence of the root-rot disease in certain areas at the United States Field Station at Sacaton, Ariz., have been reported by King and Loomis (*4*) for the years 1921 to 1924. The experiments were continued during 1925, 1926, and 1927, the farmyard manure being applied in furrows to plots C2–17, C2–19, and C2–21, and rotted alfalfa to plot C2–23, and the alternate plots C2–16, C2–18, C2–20, C2–22, and C2–24 receiving no treatment. Seed of the Pima (Egyptian) cotton was planted in rows directly over the trenches of buried organic matter. At the end of each season, just before the frost period, the locations of all

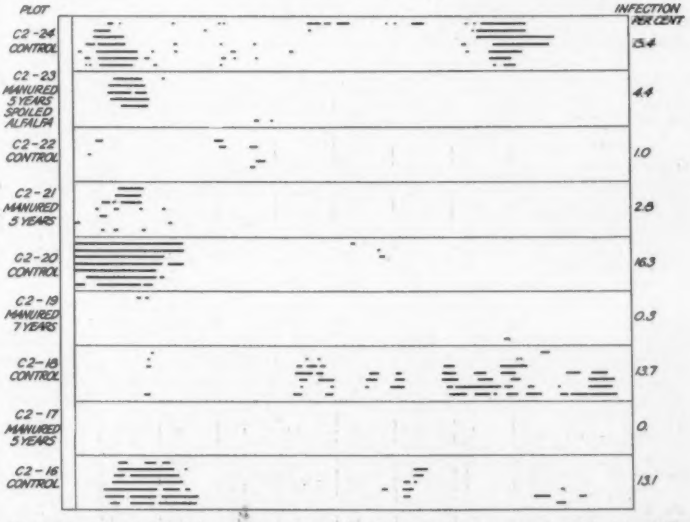

FIGURE 5.—Area infected by root rot (heavy lines) in quarter-acre experimental plots at the end of the season of 1927. Plots C2–17, C2–21, and C2–23 had received applications of organic materials for five years and plot C2–19 for seven years

dead plants were determined by measurements, and maps were prepared showing diseased areas. These maps constitute Figures 3 to 5.

At intervals of two weeks during the period when plants were being killed by the disease the number of dead plants in each plot was recorded. These data are shown in Table 1.

It will be noted from the diagrams that the infection had been reduced generally throughout all of the nine plots, but the reduction was much greater on plots where the organic manures were applied. As previously reported (*4*), the extent of infection in C2–17 in 1922 included 65.6 per cent of the area, and the infection in C2–19 in 1919 included 71.7 per cent of the area. In 1927 plot C2–17 was entirely free from the disease after five years of the manure treatment, and only 0.03 per cent of the area of plot C2–19 was infected after seven years of treatment.

TABLE 1.—*Number of cotton plants killed by the root-rot disease during intervals of two weeks on ¼-acre manured and control plots* [a] *at the United States Field Station, Sacaton, Ariz., in 1925, 1926, and 1927*

Year, plot, and treatment	Prior to Aug. 1	Aug. 15	Sept. 1	Sept. 15	Oct. 1	Oct. 16	Oct. 22	Nov. 10	Total dead plants
1925									
C2-24, control	258	612	438	354	254	45	.....	.....	1,961
C2-23 (manured [b] 3 years)	15	32	24	57	12	11	.....	.....	151
C2-22, control	52	131	136	119	76	10	.....	.....	524
C2-21 (manured 3 years)	3	13	26	83	37	3	.....	.....	165
C2-20, control	52	152	147	141	88	13	.....	.....	593
C2-19 (manured 5 years)	10	3	6	24	7	2	.....	.....	52
C2-18, control	76	224	188	166	75	40	.....	.....	769
C2-17 (manured 3 years)	3	12	15	36	15	7	.....	.....	88
C2-16, control	83	209	206	202	112	82	.....	.....	894

Year, plot, and treatment	July 27	Aug. 10	Aug. 24	Sept. 8	Sept. 22	Oct. 7	Oct. 22	Nov. 10	Total dead plants
1926									
C2-24, control	17	89	116	171	330	129	28	34	914
C2-23 (manured [b] 4 years)	1	18	14	9	55	50	5	1	153
C2-22, control	12	22	36	50	75	65	11	12	283
C2-21 (manured 4 years)	5	10	19	8	84	26	11	2	165
C2-20, control	15	114	182	111	162	37	7	3	631
C2-19 (manured 6 years)	0	1	1	2	8	5	0	0	17
C2-18, control	42	81	89	82	180	74	23	10	581
C2-17 (manured 4 years)	3	2	2	1	11	18	2	0	39
C2-16, control	17	54	107	104	268	91	46	15	702

Year, plot, and treatment	July 28	Aug. 11	Aug. 25	Sept. 8	Sept. 23	Oct. 6	Oct. 20	Nov. 3	Total dead plants
1927									
C2-24, control	13	27	41	84	121	67	60	0	413
C2-23 (manured [b] 5 years)	12	14	20	30	31	7	4	0	127
C2-22, control	2	2	0	5	6	5	25	0	45
C2-21 (manured 5 years)	1	0	1	9	26	11	14	0	62
C2-20, control	4	3	35	164	139	33	11	0	389
C2-19 (manured 7 years)	0	0	0	0	1	3	3	0	7
C2-18, control	46	43	36	92	78	57	49	0	401
C2-17 (manured 5 years)	0	0	0	0	0	0	0	0	0
C2-16, control	28	23	58	82	108	68	32	3	402

[a] The number of plants per plot was approximately 2,600. [b] Spoiled alfalfa.

The breaking up of infected areas without the influence of cultural treatment in places where the disease has been more or less continuous for several years is strikingly shown by the behavior on plot C2–24 during the years 1925 to 1927. This large area of almost solid infection began to break up in 1926, and in 1927 the dead plants were widely scattered except near the extreme ends, where solid spots of considerable size remained. The same phenomenon is also shown in plot C2–22, where in 1919 nearly 70 per cent of the total area was diseased (*4*) and in 1927 only 1 per cent. (Fig. 4.)

There appears to be no reason to doubt that the treatment with organic manures was effective in reducing plant losses. (Fig. 6.) Also, it may be considered that the reduction of infection on the treated plots had some effect in causing the general reduction throughout the entire series. In most cases where observations have been made over a long period of years with no control measures attempted there has been a tendency for the disease to become more extensive

and more damaging in infected areas that are planted successively to cotton. In the nonirrigated cotton areas the proportion of the plants killed each season may be very variable because of the varying summer

FIGURE 6.—A, Effect of manuring on the occurrence of root-rot disease in cotton. The 7-row plot in the foreground had received no manure, while the plot on the right was manured for four years and the plot on the left for six years. Note the effectiveness of the outside rows of the treated plots as barriers against invasion of the disease from the outside. B, Effectiveness of manure applied in trenches as a barrier against the spread of the root-rot disease. All of the plants in the two rows in the right foreground are dead over a length of 100 feet, while the plants in the adjoining row on the left, where the manure had been applied, remained healthy

rainfall, but as a rule the disease becomes more destructive the longer it has access to a susceptible crop. The gradual decrease in the extent of disease in the entire area, including treated and control plots, is shown in Table 2.

TABLE 2.—*Average percentage of area infected with the root-rot disease on alternate control and manured ¼-acre plots at the United States Field Station, Sacaton, Ariz., 1919–1927*

Plots	1919	1920	1921	1922	1923	1924	1925	1926	1927
Control plots: C2–16, C2–18, C2–20, C2–22, and C2–24	59.7	57.3	32.2	44.3	23.3	28.8	30.1	27.8	11.9
Manured plots: C2–17, C2–19, C2–21, and C2–23	-------	-------	*13.8	*14.1	10.0	8.5	5.0	3.8	1.9

* Only C2–19 treated in 1921 and 1922.

The highest percentage of infection occurred in 1919, when 59.7 per cent of the area was involved. In 1927 only 11.9 per cent of the total area in the five control plots was affected by the disease and only 1.9 per cent of the area in the manured plots. It seems reasonable to suppose that restricting the growth of the fungus in part of the plots, which were only 26.5 feet in width, also resulted in restricting the progress of the mycelium into new ground where it would have been more destructive. Apparently by confining it to rather definite bounds a reduction in losses was obtained.

It is apparent from the data shown in Table 1 that the disease was later in effecting injury to the cotton plants in plots treated with organic manures than where no treatment was given. In 1926 only 9 plants had died on the four manured plots prior to July 27, while 103 plants had died on the five control plots.

Considering our present knowledge of the rate of spread of the disease in cotton fields (*3*, *5*), which in Arizona varies between 12 and 20 feet per season, it is interesting to note the manner in which the advance of the disease was halted when it reached the outside rows of the manured plots. (Fig. 6.) From Figure 5, showing the extent of the infection in 1927, it will be noted that row 7 on plot C2–17 was exposed to nearly 100 feet of infection on the adjacent row of control plot C2–18, less than 4½ feet away, yet no plants on row 7 died. Row 1 on the same plot was exposed to about 20 feet of infection from the adjacent row on control plot C2–16, but there was no evidence of injury to the plants on row 1. On C2–19 the only infection that occurred during the years 1926 and 1927 was located on outside rows 1 and 7 (figs. 4 and 5), which indicates that the mycelium invaded these rows from the control plots but was unable to make rapid progress. A number of excavations were made in plots C2–17 and C2–19 at the end of the season in 1926 and 1927 to determine whether any of the roots of the plants, apparently healthy, had been attacked by the fungus, but all of them were free from root-rot lesions and mycelium. It appears, therefore, that the disease which was once very prevalent in these two plots had been practically eradicated.

TRENCHING EXPERIMENTS

In the effort to determine the method by which the organism moves through the soil in its circular advance from a center of infection, a trench about 20 inches deep was dug about 18 inches in advance of the circumference on one side of a rapidly spreading root-rot spot in an alfalfa field on August 22, 1927. About three weeks after the trench was dug several spore mats appeared on the outside vertical wall near the bottom of the trench. (Fig. 7.) A few days

FIGURE 7.—Conidial mats of *Phymatotrichum omnivorum*, showing as a line of irregular white spots along the bottom of the outside wall of a trench surrounding a root-rot circle in an alfalfa field. This trench, which was dug 20 inches deep and 18 inches in advance of the dying plants, was only partially effective as a barrier, since the mycelium apparently passed under it in two places

later, following a rainy period, the bottom and sides of the trench for several feet became lined with conidial mats. Peltier, King, and Samson (*6*) showed that the mycelium was seldom found on the roots of healthy alfalfa plants more than 12 inches in advance of the ring of recently wilted alfalfa plants. Since the outside wall of the trench was more than 2 feet beyond the ring of wilted plants, it seems probable that the organism was able to pass under the trench. From near-by excavations it was observed that the mycelium often covered the roots at a depth of 30 inches underground.[4] Thus it seems probable that some of the mycelium below the 20-inch level may have extended through the soil under the trench and carried the infection to the outside of the barrier. At the end of the season two small spots of infection were found on the outside of the trench, but at the ends of the trench the disease had advanced much farther; hence it was evident that the trench had delayed its progress.

DORMANT CENTERS OF INFECTION

So far there has been no evidence to indicate that root-rot infection could be started in new areas by the dissemination of spores, although spores are produced in great abundance in some places. There has been evidence, however, that infected spots may disappear for a period and then reappear in the same location in later years. This occurrence was illustrated on plots C3–5, C3–6, and C3–7. In 1920 and 1921 the infected areas on these plots which were planted to cotton were charted. (Figs. 8 and 9.) In 1921 several scattered spots on the north end of these plots which were mapped in 1920 did not reappear. During the period 1922 to 1925 the plots were planted to nonsusceptible crops—grain, corn, and sorghums. Two crops were grown each year, which required breaking the land twice a year, in addition to several cultivations that the corn and grain sorghums received. In 1926 the area was again planted to cotton, and the root-rot injuries were charted at the end of the season. (Fig. 10.) Since this infection was not extensive, it was decided to replant to cotton in 1927. The disease was much more destructive in 1927, and in addition to the areas being more extensive than in 1926, small spots appeared where no injuries had appeared in the previous season. (Fig. 11.) This was shown on the north end of plots 5, 6, and 7, where no infection was present in 1921 or 1926, but several spots occurred in 1927. By referring to Figure 8 it will be seen that the area occupied by these spots was infected in 1920. A somewhat similar behavior occurred on plots C3–10 and C3–11, which were also cropped to cereals and sorghums during the years 1922–1925. Several isolated spots appeared at the north end of these plots in 1927 which were not apparent in the cotton planted in 1926, but which were in close proximity to the location of spots that had been mapped in 1921.

Excavations were made in one of these spots in December, 1927, to determine whether any living root fragments remained in the soil from previous crops. At a depth of 18 inches pieces of live roots from the 1926 plants were found, but for the most part the roots of previous crops were badly disintegrated. Some old strands of myce-

[4] In 1928 rotted roots of pistache and almond trees, covered with root-rot strands, were found in trenches at a depth of 5 feet 8 inches.

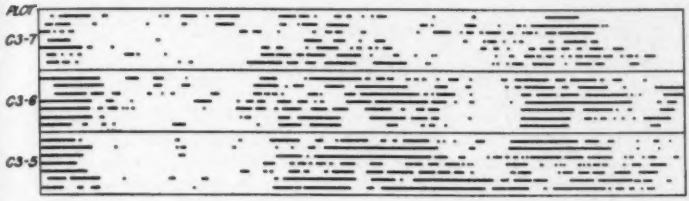

FIGURE 8.—Extent of root-rot infection (heavy lines) in cotton on quarter-acre plots C3-5 to C3-7 in 1920

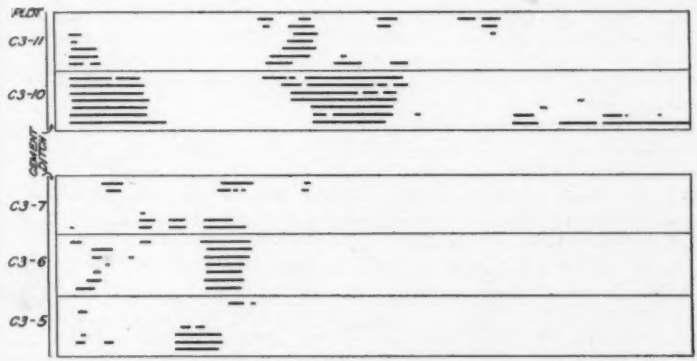

FIGURE 9.—Extent of root-rot infection (heavy lines) in cotton on quarter-acre plots C3-5, C3-6, C3-7, C3-10, and C3-11 in 1921. It will be observed that the infection in plots C3-5, C3-6, and C3-7 was much less than in 1920 as shown by Figure 5

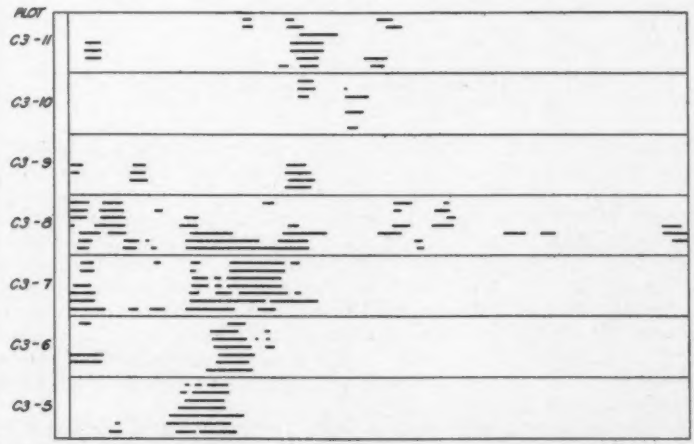

FIGURE 10.—Recurrence of root-rot infection (heavy lines) on quarter-acre plots C3-5 to C3-11 in 1926 after being planted to nonsusceptible crops for four years

lium were found on the old decayed roots and also on the partly decayed roots of the 1927 plants, but they appeared to be dead, and no active mycelium could be found at this time.

INFECTION DEEP-SEATED IN SOME AREAS

The possibility of the fungus being able to exist for long periods at considerable depth was suggested by the reappearance of spots in which the disease had been dormant for some years. That the particular zone of soil in which the disease is most active might vary with the character of the soil and climate was suggested by W. T. Swingle and O. F. Cook. Both predicted that the active zone of the disease recently discovered in a new district in California would likely prove to be deep-seated, on account of the sandy character of the soil and its properties of absorbing heat to considerable depth.

FIGURE 11.—Recurrence of root-rot infection (heavy lines) on quarter-acre plots C3–5 to C3–11 in 1927, the second year in cotton after four years in nonsusceptible crops. Note especially the reappearance of diseased spots in sections of the plots C3–5 to C3–7 where no plants were killed in 1926

Investigations made in December, 1927, in a cotton field in this area where only three cotton plants died during the season proved that this was the case. All of the cotton plants for 10 feet or more surrounding the dead plants were found to be infected, although they showed no evidence of injury aboveground. In almost every case that portion of the taproot extending below the collar for 5 to 10 inches remained uninjured (fig. 12, A), although in some cases mycelium was found on the healthy tissues. Below this the roots showed injuries in varying degrees, but on most plants a section of the taproot 5 to 15 inches in length was completely rotted. Some of these plants with a part of their taproots destroyed had sent out bunches of fibrous roots above the injured area. (Fig. 12, B.)

The activity of the disease at such depths offers a further complication in accurately mapping the extent of the infected areas. It is obvious that under such conditions the extent of the disease is not indicated by the position of the plants that are killed.

CONIDIAL MATS IN ABSENCE OF DEAD PLANTS

Thornber (*9*), King (*3*), and others have reported that spore mats are most commonly found in close proximity to plants that have recently died from the root-rot disease. In 1919 one of the writers observed a large spore mat on an irrigation-ditch bank beneath a large cottonwood tree. The tree appeared to be healthy, and the only other vegetation near by was Bermuda grass, which was also healthy in its appearance. The nearest plants that indicated the presence of the root-rot organism were some dead cotton plants about 40 feet away.

In 1927 a field of young alfalfa near Sacaton showed many spots of root-rot infection. The soil was extremely fertile and contained a large quantity of organic matter from decaying roots of native plants and buried cotton stalks of a previous crop. Throughout the summer of 1927 spore mats could be found at almost any time in this field, but they were especially abundant after rainy or humid periods. (Fig. 13.) In a great number of cases large spore mats could be found surrounded by healthy plants and at considerable distance from any dead plants. Since this behavior was unusual, the locations were noted, and later observations showed that the alfalfa plants soon began to die in these areas. Upon digging beneath the spore mats in such locations, large fragments of buried and partly decayed cotton stems or roots were frequently found, and these were invariably densely covered and pervaded with Phymatotrichum strands. (Fig. 14, B.)

GROWTH AND DEVELOPMENT OF CONIDIAL MATS

From the behavior of the root-rot fungus when opportunity is afforded for fruiting, it is obvious that the distribution of the mycelium throughout the upper layers of soil is extensive and capable of rapid growth when conditions are favorable. Within a few hours after a summer rain in the vicinity of Sacaton, Ariz., the mycelium in many of the recently infected areas in alfalfa fields rapidly pervades all portions of the soil near the surface and begins to develop an arachnoid structure of whitish mycelium on the surface of the soil about the plants that have recently died. (Fig. 13, A.) This type of mycelium grows rapidly outward from the center where it begins, if conditions remain favorable, and it sometimes covers an area of a square foot or more within 48 hours. The formation of a compact mass of mycelium and conidiophores in a definite palisade structure from 3 to 10 millimeters in thickness rapidly follows the advance of the arachnoid type and develops on top of the thin stromalike structures which form on the surface of the soil. It is not infrequent that several ounces of fungous material is developed on a square foot of soil surface within 8 or 10 hours.

The progressive growth of a small mat is shown in Figure 13. Under some conditions the growth is much more rapid and covers a larger surface than is shown in this instance.

The occurrence of conidial mats in great abundance in areas where a large quantity of organic matter is present in the soil and the rapid rate at which they grow in such places suggest that the organism may derive at least a part of its nourishment from the dead organic material in the soil.

The resistant character of the mycelium which takes part in the development of the spore mats was shown by observations made in the summer of 1927. The bottom and sides of portions of a narrow trench dug for drainage purposes around a large haystack near Sacaton was found to be almost completely lined with large mats on August 16. A period of dry weather followed, and conditions were

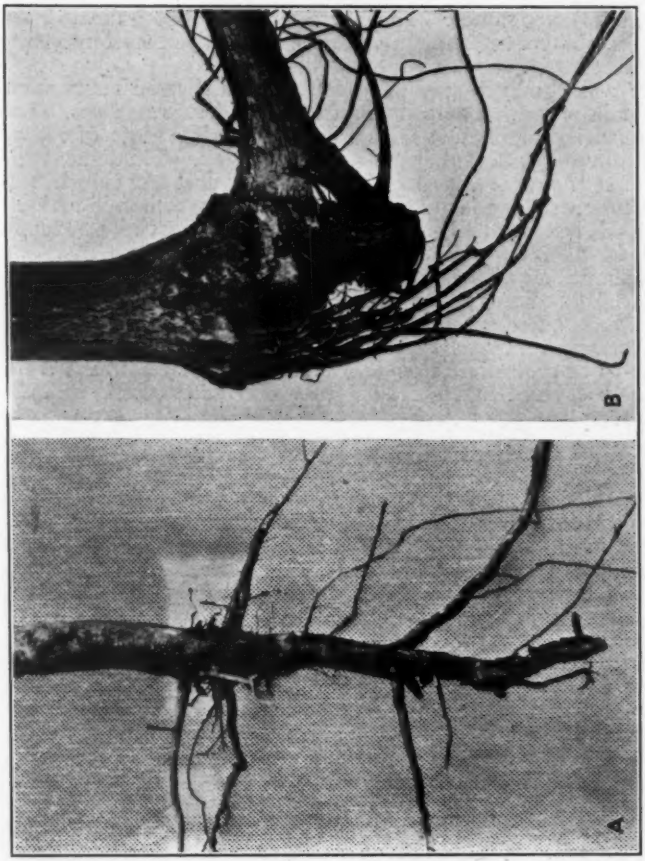

FIGURE 12.—A, Root-rot injury to cotton plant several inches below the surface of the soil. Many large plants in fertile soil are able to survive such injuries for long periods. Under some conditions the mycelium is not destructive to the roots near the surface and large numbers of plants are able to live through the season, although some of the deeper roots are destroyed. B, Fibrous roots produced by cotton plants after the taproot has been destroyed by the root-rot fungus. Note the lateral root at the right which became enlarged because of the injury to the other roots, being responsible for the plant remaining alive until the end of the season

not again favorable for fruiting until about September 14. At that time the trench was again examined for spore mats, and it was found that many of the old mats, about 29 days old, although badly weathered and disintegrated near the center, had begun new growth on the outer margins, and a whitish elevated band of new material had been formed. A similar occurrence of this revived growth was observed in mats that developed about 18 inches underground in a runway made by rodents. This behavior seems to indicate that in protected locations the fruiting mycelium is capable of existing in a somewhat

FIGURE 13.—Progressive stages in the development of a conidial mat on the periphery of a circle of dying plants in an alfalfa field. A, Photographed at 3 p. m., Sept. 14. Tiny mat 1 day old showing surrounding expanse of filamentous mycelium composed of a large-celled hyphae. B, Photographed at 8 a. m., Sept. 15. Note the growth that occurred during the 17-hour interval. C, Photographed at 8 a. m., Sept. 16. Note how the mat has changed color and become depressed in the center. The growth had practically ceased at this time. (Natural size)

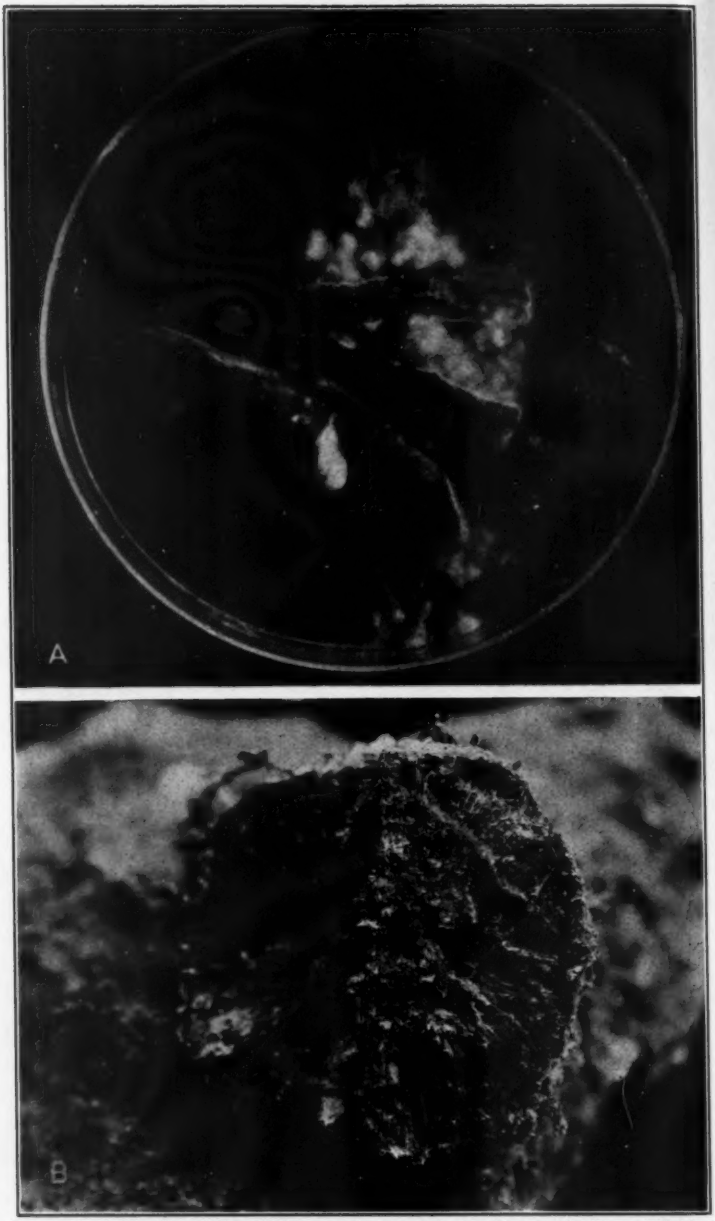

FIGURE 14.—A, Mycelium of *Phymatotrichum omnivorum* growing in culture on old cotton roots that had been buried in the soil for two years after being cut off by the plow. Note the numerous pseudosclerotia that have developed, indicating that the mycelium has been able to draw nutrients from the decayed material. (Natural size.) B, Section of a decayed cotton stalk broken in two to show a large strand of mycelium that had entered through a crack. The stalk had been more than two years below ground. × 5

dormant condition over long periods and is able to begin new growth when a favorable opportunity is afforded.

The development of spore mats in holes and trenches from 1 to 2 feet below the surface of the soil [5] also shows that the distribution of the organism in the soil is extensive and that the fungus is able to take advantage of any favorable situation in which to develop its fruiting bodies.

ARTIFICIAL CULTURES

The root-rot fungus is readily isolated and can be grown on various kinds of culture media. Taubenhaus and Killough (*8*) and Peltier, King, and Samson (*6*) have described methods by which isolations can be made. The essential feature of the methods consists in dissecting bits of tissues from just beneath the epidermis on the edge of young lesions on the roots of plants that have been recently attacked. These bits of infected tissue are dipped in a solution of mercuric chloride, 1 to 1,000, for a few seconds, and the excess solution is removed by rinsing them with sterile water or by absorbing the solution between sterile filter papers. The bits of tissue are then dropped on sterile plugs of cotton roots or other nutrient material in test tubes.

In preparing pure cultures for use in inoculation experiments, quart fruit jars were filled with sand or soil and 2-inch sections of cotton roots in alternate layers. After the contents were well moistened with distilled water and the modified caps stoppered with cotton, they were sterilized, and transfers from pure cultures in tubes were added. In a few weeks the contents of many of these jars become permeated with mycelium. In a large proportion of these jars numerous pseudosclerotia were formed, some of which were more than 1 centimeter in diameter. Many small cavities in the sand or soil became completely filled with these pseudosclerotia, whose connection with the large strands was easily determined by examining those on the glass surfaces. In some cases the pseudosclerotia were definitely aerial and were formed on the strands that had grown along the glass above the soil and cotton roots.

It was observed that when the contents of the jars were saturated with moisture no growth of mycelium occurred except on the sections of roots that projected out of the soil. Also when the moisture in the jars became so reduced that no condensation occurred on the glass near the top, it was noted that the growth of the mycelium was restricted. This behavior agrees with field observations in that the moisture content of the soil under natural conditions has an important influence on the growth of the fungus.

When parts of dead cotton roots that had been turned under the soil and had remained buried for two years were dug up, sterilized, and used in the jar cultures, the growth of the mycelium was rapid and profuse, and pseudosclerotia were produced as in the cultures with fresh roots. (Fig. 14, A.) Among the kinds of nutrients on which the fungus was grown successfully were fresh roots of cotton, alfalfa, cowpeas, peach, Lycium, pecan, and dead roots of cotton and alfalfa (fig. 15) 1 and 2 years old. Cultures of the fungus from dead cotton and Lycium roots are shown in Figure 15. Also it was grown suc-

[5] Spore mats were observed in August, 1928, in trenches at a depth of 5 feet from the surface of the soil.

cessfully on agar media containing decoctions of alfalfa, cotton, beans, prunes, and corn meal, and on cottonseed and wheat kernels. Pseudosclerotia were formed in a majority of the cultures with all of these media. The behavior of the fungus when limited to a nutrient of dead roots indicates that it may be able to exist for long periods under

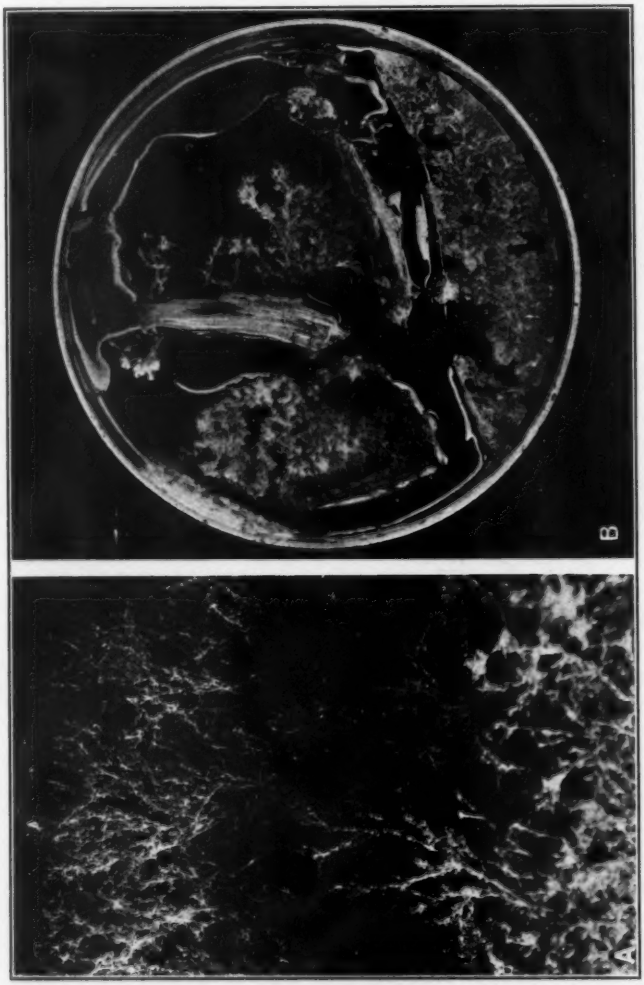

FIGURE 15.—A, Mycelium of *Phymatotrichum omnivorum* in pure culture growing on dead cotton roots on a thin layer of prune agar in a Petri dish. Note the thick strands growing from the dead root (out of focus at the bottom of the photograph). The root sections were obtained from several inches beneath the surface of the soil where they had been cut off by the plow and turned under for several months. × nearly 5 diameters. B, Growth of *Phymatotrichum omnivorum* on pieces of dead root of *Lycium* sp. on prune agar. The roots became densely covered with the mycelium before it extended to the thin layer of agar. The irregularities in the agar were due to its becoming dried out. A number of small pseudosclerotia are apparent on the mycelium. (Natural size)

natural conditions as a saprophyte. (Fig. 16.) This is in accord with findings of George T. Ratliffe (7) at the San Antonio Field Station in Texas, who found the organism existing on fragments of mesquite roots that had remained under the soil for many years. Not infrequently, alfalfa roots killed by plowing are found long afterward supporting live mycelium. (Fig. 16.)

FIGURE 16.—Coarse strands of root-rot mycelium on an old dead alfalfa root. × 5

INOCULATION EXPERIMENTS

The successful inoculation of healthy cotton plants with *Phymatotrichum omnivorum* has been reported by King (*1*), by Taubenhaus and Killough (*8*), and by Peltier, King, and Samson (*6*).

Further experiments in producing infection were conducted during the latter part of the season of 1927 at Sacaton. The methods employed were essentially the same as those described by King (*1*), in which the taproots of normal plants in uninfected locations were exposed in narrow trenches and the inoculum placed in contact with the healthy roots. The experiments in 1927 demonstrated that inoculations can be made without great difficulty when certain conditions are favorable.

In trench No. 1 the roots of 14 Pima cotton plants of average size were exposed, and sections of roots from recently infected plants were placed in contact with them on September 12. The inoculum was held in place with moist cotton lint, and the trench was refilled with soil. On September 27 one of the inoculated plants wilted suddenly, and two days later two others showed the characteristic symptoms of root-rot attack. (Fig. 17, A.) However, the field was irrigated about this time, and one of the wilted plants recovered and showed no further distress during the season. The second plant that died was dug up, and from it the root-rot fungus was isolated and grown in cultures. None of the other 11 plants exhibited any effects from the treatment, but when they were dug up and the roots examined on December 18 it was found that all of them had been attacked to some extent by the fungus. On several of them a part of the taproot had been completely destroyed, but the plants had been supported by large laterals which were above the infected zone.

In trench No. 2 the roots of 12 very large plants, more than 6 feet in height, were exposed on September 13. Six plants on one side of the trench were inoculated with sections of roots recently attacked by the disease under field conditions, and the six plants on the other side were inoculated with 2-inch sections of cotton roots from pure cultures grown in fruit jars. The sections of infected roots were placed in contact with the taproots of the healthy plants about 4 inches below the surface of the ground. On the twenty-first day after treatment one of the plants inoculated with this pure-culture material wilted slightly and remained in a semiwilted condition for several days. Irrigation water was applied at this time, and the plant recovered and remained healthy until the end of the season. On December 18 the plants were exhumed and their roots examined. It was found that the taproots of all of them were attacked at a distance of 4 or 5 inches below the crown, and in some cases the roots were rotted to a depth of 12 to 14 inches in the soil. The large laterals served to sustain the plants, as only a few of these were injured. When examined in December the healthy surface area of the taproots above and below the rotted area and the surface of many of the laterals were covered by live strands of the root-rot fungus. In some cases the strands had extended 5 or 6 inches beyond the zone of the nearest lesion. This behavior of the mycelium seems to be characteristic during the cool-weather periods in the fall and spring, when the disease is not very active. If these inoculations had been made during the early part of the summer, when the fungus is ordinarily more active, it is believed that a greater

FIGURE 17.—A, Cotton plants dying after being inoculated with the root-rot fungus in an area where the disease had never existed. B, Method of attempts to transmit the root-rot disease to cotton plants grown in drums by use of cylindrical cores of earth from infected spots where the disease was active and fruiting. These efforts in 1927 were unsuccessful

number of the injured plants would have died.[6] Even during the summer months, however, roots are sometimes found on which the mycelium is abundant before any lesions or depressions in the root surface appear.

It is apparent from these experiments that infection can be produced in soil areas where the disease has never been in evidence, but the time of the season, the size of the plants and their root systems, and the moisture conditions seem to have an important relation to the progress of the new infection.

Other attempts were made to transmit the disease to healthy cotton plants grown in tanks and drums by inserting cylindrical cores of soil from the active margin of dying spots of alfalfa into the soil adjacent to the plants in the tanks and drums. For the large tanks, cores of soil 12 inches in diameter by 12 inches long with fresh undisturbed spore mats on the upper surface were removed from an infected alfalfa area by the use of date-tree transplanters and inserted in the soil in the tanks between two cotton plants. Smaller cores about 6 inches in diameter by 8 inches long with spore mats on the top surface were used as inoculum for the plants grown in drums. None of the cotton plants died as a result of this treatment, but when the cores of soil were dissected in December live mycelium was still in evidence on partially decayed pieces of alfalfa root. There was no evidence, however, of the mycelium having extended into the soil surrounding the cores, and the greater part of the mycelium was dead and brittle.

SUMMARY

Further studies on the cotton root-rot disease at the United States Field Station, Sacaton, Ariz., have shown that the extension of infection into new areas can be effectively controlled by treating the soil with solutions of formaldehyde and cresylic acid.

Experiments in which organic manures were applied in furrows to alternate ¼-acre plots continuously cropped to cotton for several years show that this treatment is effective in reducing the extent of infection, in delaying the appearance of the disease, and in retarding its injuries to the plants.

The manured plots served as effective barriers in restricting or retarding the advance of the mycelium from adjacent untreated areas.

Plot C2–17, in which 65.6 per cent of the total area was infected in 1922, was entirely free from the disease in 1927 after five years of manuring, and plot C2–19, in which 71.7 per cent of the area was infected in 1920, showed only 0.03 per cent infection in 1927 after seven years of manurial treatment.

The breaking down or dissipation of infected areas where practically all of the cotton plants have died for several years without the influence of any cultural treatment is clearly shown by maps which define the location of the infection over a number of years.

It was indicated that under Arizona conditions the fungus was capable of passing under an open trench 20 inches deep which was dug in advance of a ring of dying alfalfa plants.

[6] Of 55 cotton plants inoculated July 20, 1928, with one or two 4-inch sections of diseased roots inserted in the soil in contact with the taproot, 15 died within 10 to 25 days and transmitted the disease to adjacent plants. Six plants grown in tanks and drums and inoculated in late July and early August with pure cultures grown in quart fruit jars died within 5 to 19 days, and the disease spread and killed adjacent plants.

The occurrence of conidial mats in areas where no dead plants were evident was verified, and there appeared to be a relationship between these mats and buried fragments of decaying roots beneath them.

A study of maps which define the location of the disease on the same plots over a number of years shows that spots of disease may disappear and recur after a number of years as isolated centers of infection and progress as before.

Further attempts at inoculating healthy cotton plants in fields where no root rot had previously appeared were successful in producing infection on the roots, though only a few of the plants were killed by the disease. This was probably due to the lateness of the season at the time inoculations were made and to the influence of irrigation water on the small mass of mycelium present.

Efforts to transfer the infection to cotton plants growing in large tanks and drums by transferring cylindrical cores of infected soil on which the fungus was fruiting were unsuccessful.

It was found that the root-rot organism is readily isolated and can be cultured successfully on various kinds of roots and other media. Its ability to grow on dead roots indicates that it may exist in nature as a saprophyte and may be able to live in the soil in the absence of live root tissues.

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GRAIN LOSSES IN FEEDING CORN SILAGE TO DAIRY COWS[1]

By R. B. BECKER, formerly *Associate Professor of Dairying*, and WILLIS D. GALLUP, *Assistant Chemist, Oklahoma Agricultural Experiment Station* [2]

INTRODUCTION

Dairy cows are able to digest only a portion of the grain in cane and kafir silage and a considerable amount passes into the manure as whole kernels. The practical significance of this loss, which may be attributed to the toughness and small size of the kernels, has been emphasized in a previous publication (*2*)[3]. Indications of similar losses were observed with dairy cows that received corn silage. This observation led the authors to investigate the grain losses that actually occur when corn silage is fed to dairy cows.

REVIEW OF LITERATURE

Utilization of whole corn in silage has been investigated briefly by LaMaster and Morrow (*10*), who made their determinations upon small samples of silage and samples of manure collected from two cows during a 5-day period. The amount of whole corn in the manure, as observed, constituted 1.86 per cent of the grain in the silage. This represents a loss which is relatively small when compared with similar losses (*12*) of shelled corn which amount to 22.75 per cent with mature dairy cows, 10.77 per cent with yearling heifers, and 6.28 per cent with calves. Wilbur (*5, p. 33*) found the losses of shelled corn when fed to dairy cows to be between 30 and 35 per cent, while losses of cracked corn were only between 5 and 10 per cent.

Losses which represent 33.91 and 49.46 per cent of the grain in cane and kafir silages, respectively, were observed (*2*) in the manure of dairy cows. These losses (*3*) amounted to 9.41 per cent of the calculated total digestible nutrients in kafir silage and 7.13 per cent of those in cane silage. Utilization of the whole grain in kafir silage was from 9 to 13 per cent greater than that of threshed, soaked, and head kafir grain fed to 2-year-old steers (*9*). LaMaster and Morrow (*10*) reported 27.55 per cent of the whole grain in cane silage voided in the manure of dairy cows. Cave and Fitch (*4*) obtained results based on counted kernels in small samples of material, which indicated a loss as great as 90 per cent of the grain in cane silage.

Corn undergoes a loss in the crib as well as in the silo. Corn stored from January 5 to June 1 (147 days) lost 5.6 per cent in weight (*6*). Gaines (*7*), from an analysis of grain at the time of harvest, found that corn in the crib lost 2.25 per cent, while kernels in corn silage lost 5.08 per cent of the dry matter. Part of the crude protein

[1] Received for publication Apr. 2, 1929; issued August, 1929.
[2] Acknowledgement is made to the late Prof. A. C. Baer, and to P. C. McGilliard of the dairy department, who instigated the experimental work comparing corn silage with mangels in dairy rations. Data presented here were derived from cows on this investigation. Kenneth Corbett cared for the cows used in this feeding trial.
[3] Reference is made by number (italic) to "Literature cited," p. 227.

and ash of corn kernels (*11*) was taken up by the plant juices in the silo, thereby increasing the protein content of the leafy portion of the silage. Bailey (*1*) showed the shrinkage in shelled corn to be due to loss of moisture and carbon dioxide in the respiration of the dormant seed.

There is need of data from more animals over a longer feeding period, which takes separate account of the whole and cut kernels, on which to base a calculation of the losses of corn grain in the silo, and later in the digestive system of the dairy cow.

EXPERIMENTAL METHODS

Four cows on a 20-day feeding trial (10 days preliminary and 10 days experimental) were given a daily ration consisting of 30 pounds of corn silage and 10 pounds of alfalfa hay per 1,000 pounds live weight, and, in addition, mixed grain consisting of wheat bran, ground oats, corn meal, and choice cottonseed meal to meet the Morrison (*8, p. 746*) feeding standard. Reid Yellow Dent corn cut in the glazed stage of maturity and ensiled in a hollow-tile silo furnished the only source of whole-kernel corn. Samples of the ear corn procured at time of filling the silo were cured on the cob, shelled, and analyzed with the samples mentioned below.

The usual precautions (*2*) were observed in caring for the animals and recording the amounts of feed eaten and refused. A 10-pound sample of silage was taken from the silo each day during the experimental period. Corn kernels were separated from the silage by hand, weighed, and sampled. The entire amount of corn in any refused feed and in the manure of each cow was separated out daily by use of water and screens. This grain was air dried, weighed, and sampled

Chemical analyses were made of the shelled corn, corn silage, and grain from the silage and from the manure. The proportion of whole and cut kernels was determined by separating and weighing these fractions from large composite samples of the grain obtained from the silage and from the manure.

PRESENTATION OF DATA

During the 10-day experimental period the four cows consumed 1,335 pounds of corn silage and refused 25 pounds. The ten 10-pound samples of fresh silage contained 24.95 pounds of corn grain. Allowing for 0.78 pound of air-dry grain in the refused silage, the four cows consumed 170.77 pounds of dry matter as grain in the corn silage. By the method described 15.89 pounds of air-dry corn grain (14.48 pounds of dry matter) were recovered from the manure. This loss constituted 8.47 per cent of the grain contained in the silage.

Whole kernels made up 67.87 per cent by weight of the corn grain in the silage. Likewise, 40 per cent of the grain voided in the manure was whole kernels. By applying these percentages, it was calculated that 4.36 per cent by weight of the whole kernels in silage was voided as whole kernels in the manure. It was noticed repeatedly that the starchy portion of numerous cut and broken kernels had been digested, leaving large fragments of corn bran in the manure.

Records of silage consumed and of grain recovered from the manure of individual cows are presented in Table 1.

TABLE 1.—*Amount of grain in corn silage fed to dairy cows during 10 days and of air-dry corn recovered from the manure*

Cow No.	Silage offered	Silage refused	Moist corn in silage	Air-dry corn in refused feed [1]	Air-dry corn recovered from manure [1]
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	300	21	74.85	0.28	2.42
2	360	0	89.82	0	6.42
3	360	0	89.82	0	4.04
4	340	4	84.83	.50	3.01

[1] Since water was used in separating grain from manure and refused feed, it became necessary to report these data on the air-dry basis.

Chemical analyses of the original shelled corn, fresh silage, grain from the silage, and air-dry grain recovered from the manure are presented in Table 2.

TABLE 2.—*Composition of original corn kernels, corn silage, and grain from silage fed and of grain recovered from the manure of dairy cows during a 10-day period*

Description of sample	Moisture in original material	Composition of dry matter				
		Crude protein	Ether extract	Nitrogen-free extract	Crude fiber	Ash
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Shelled corn cured on the cob	9.10	9.62	4.27	82.30	2.53	1.28
Whole kernels from silage	49.46	7.14	4.93	84.65	2.38	.90
Whole kernels from manure, air-dry	8.74	6.62	4.36	85.59	2.63	.80
Total corn from silage	49.46	6.95	4.67	84.72	2.53	1.13
Total corn from manure, air-dry	8.90	6.26	2.87	87.30	3.13	.44
Corn silage	69.26	8.31	3.45	66.51	17.47	4.26

The changes in composition of the corn grain while in the silo and in contact with the digestive juices of the cow may be obtained from these analyses.

DISCUSSION OF RESULTS

The data presented show that 8.47 per cent by weight of the corn grain fed in silage to dairy cows was voided with the manure. Only 4.36 per cent of the whole kernels in the silage was recovered as whole kernels from the manure. The loss of corn grain in silage was smaller than that with shelled corn (*5*, *12*). These losses were appreciably less than those observed (*2*) with the small, hard grains in Early Sunrise kafir and Kansas Orange cane silages. The losses of whole corn, as found by this method, are higher than those observed by LaMaster and Morrow (*10*). There is evidence to show that the corn grain recovered from the manure of these cows is of sufficient value to be salvaged profitably by swine or poultry.

The changes which occurred in the corn grain in the silo and in the cow's digestive system are evident from the analyses presented in Table 2. The apparent increase in the ether-extract content of ensiled kernels is due to the formation of ether-soluble compounds during the fermentation process in the silo. The apparent losses,

as seen in Table 3, were 25.78 per cent of the crude protein, 29.69 per cent of the ash, and 5.94 per cent of the crude fiber in the original whole kernels. Apparent gains were found of 2.85 per cent and 15.45 per cent of the nitrogen-free extract and ether extract, respectively, in the whole kernels separated from the silage. These changes were produced by bacterial and enzymatic action on constituents of the corn kernels, changing them into carbon dioxide and other by-products, some of which were ether-soluble while others appeared as nitrogen-free extract. The loss in ash may be attributed to its solubility in the plant juices, as observed by Perkins (*11*).

TABLE 3.—*Composition of corn grain from silage and from manure compared with that of shelled corn cured on the cob*

Description of sample	Crude protein	Ether extract	Nitrogen-free extract	Crude fiber	Ash
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Shelled corn cured on the cob	100.00	100.00	100.00	100.00	100.00
Whole kernels from silage	74.22	115.45	102.85	94.06	70.31
Whole kernels from manure	68.81	102.11	104.00	103.95	62.50
Total corn grain from silage	72.35	109.37	102.94	100.00	88.28
Total corn grain from manure	65.07	67.21	104.86	123.71	34.38

The corn kernels recovered from the manure had given up a small proportion of their crude protein, ether extract, and ash, and consequently showed a slight apparent gain in content of crude fiber and nitrogen-free extract after coming in contact with the cow's digestive juices. The percentage changes of these constituents in the corn grain are presented in Table 3.

By applying coefficients of digestibility (*8, p. 722–726*) to the nutrients in corn silage and in the grain voided with the manure it was calculated that 5.22 per cent of the digestible crude protein and 5.26 per cent of the total digestible nutrients in the silage were contained in the grain recovered from the manure.

SUMMARY AND CONCLUSIONS

Corn kernels lost protein, crude fiber, and ash in the silo, but gained in percentages of nitrogen-free extract and ether extract.

When dairy cows were fed silage made from dent corn in the glazed stage of maturity 8.47 per cent by weight of the grain in the silage was voided in the manure. Only 4.36 per cent by weight of the whole kernels in the silage was recovered as whole kernels from the manure. Analyses showed slight losses of protein, ether extract, and ash from the corn grain in silage which passed through the cow's digestive tract. Dairy cows utilize the whole and cut kernels in silage more efficiently than they utilize shelled corn.

The corn grain voided in the manure was calculated to contain 5.22 per cent of the digestible crude protein and 5.26 per cent of the total digestible nutrients in the corn silage.

The loss of whole corn in silage fed to dairy cows is decidedly less than that which occurs when cattle consume shelled corn. Even this small loss may be salvaged by allowing swine or poultry access to the manure.

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U. S. GOVERNMENT PRINTING OFFICE: 1929